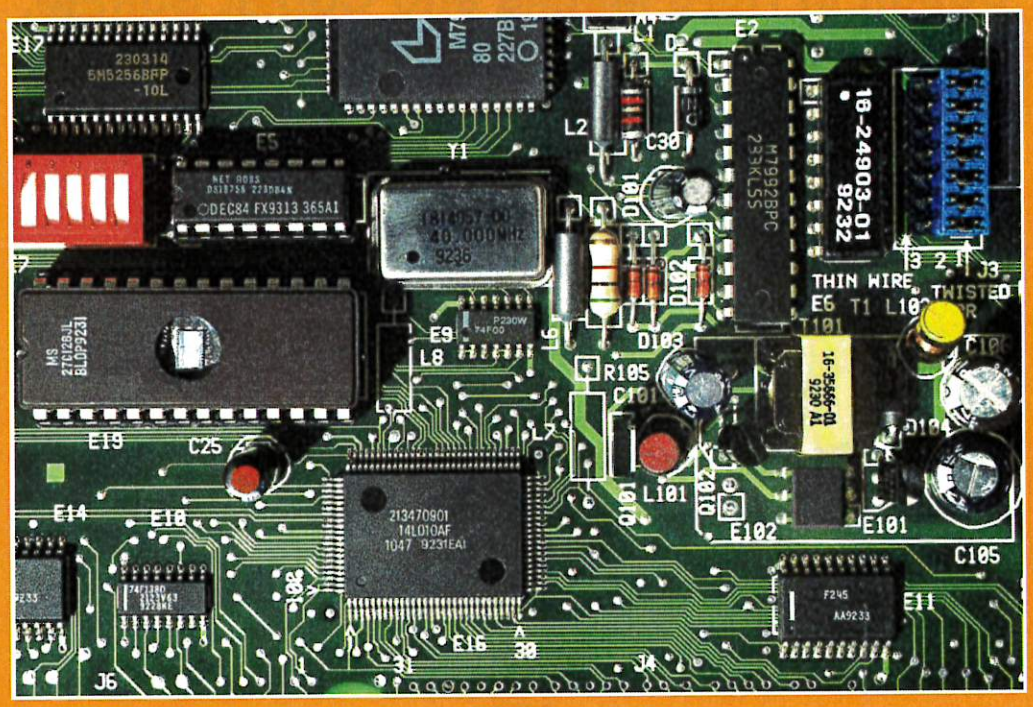
Exhibit 5

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

VOLUME 86

Cobalt in Hard Metals and Cobalt Sulfate, Gallium Arsenide, Indium Phosphide and Vanadium Pentoxide



LYON, FRANCE
2006

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IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

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This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Carcinogenic Risks to Humans,
which met in Lyon,

7–14 October 2003

2006

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, life-style factors and biological and physical agents, as well as those in specific occupations.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields; and to indicate where additional research efforts are needed.

The lists of IARC evaluations are regularly updated and are available on Internet: http://monographs.iarc.fr/

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Cover: Some metallic compounds evaluated in this volume are used in integrated circuit boards which represent a new use of these metals.

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NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean the probability that exposure to an agent will lead to cancer in humans.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Carcinogen Identification and Evaluation, so that corrections can be reported in future volumes.

IARC WORKING GROUP ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS: COBALT IN HARD METALS AND COBALT SULFATE, GALLIUM ARSENIDE, INDIUM PHOSPHIDE AND VANADIUM PENTOXIDE

Lyon, 7–14 October 2003

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PREAMBLE

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

PREAMBLE

1. BACKGROUND

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. The *Monographs* programme has since been expanded to include consideration of exposures to complex mixtures of chemicals (which occur, for example, in some occupations and as a result of human habits) and of exposures to other agents, such as radiation and viruses. With Supplement 6 (IARC, 1987a), the title of the series was modified from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* to *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, in order to reflect the widened scope of the programme.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs series*. Those criteria were subsequently updated by further ad-hoc working groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987b, 1988, 1991a; Vainio *et al.*, 1992).

2. OBJECTIVE AND SCOPE

The objective of the programme is to prepare, with the help of international working groups of experts, and to publish in the form of monographs, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* may also indicate where additional research efforts are needed.

The *Monographs* represent the first step in carcinogenic risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that certain exposures could alter the incidence of cancer in humans. The second step is quantitative risk estimation. Detailed, quantitative evaluations of epidemiological data may be made in the *Monographs*, but without extrapolation beyond the range of the data available. Quantitative extrapolation from experimental data to the human situation is not undertaken.

The term 'carcinogen' is used in these monographs to denote an exposure that is capable of increasing the incidence of malignant neoplasms; the induction of benign neo-

plasms may in some circumstances (see p. 19) contribute to the judgement that the exposure is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991a; Vainio *et al.*, 1992; see also pp. 25–27).

The *Monographs* may assist national and international authorities in making risk assessments and in formulating decisions concerning any necessary preventive measures. The evaluations of IARC working groups are scientific, qualitative judgements about the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which regulatory measures may be based. Other components of regulatory decisions vary from one situation to another and from country to country, responding to different socioeconomic and national priorities. **Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments and/or other international organizations.**

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures. A survey of users in 1988 indicated that the *Monographs* are consulted by various agencies in 57 countries. About 2500 copies of each volume are printed, for distribution to governments, regulatory bodies and interested scientists. The Monographs are also available from IARC*Press* in Lyon and via the Marketing and Dissemination (MDI) of the World Health Organization in Geneva.

3. SELECTION OF TOPICS FOR MONOGRAPHS

Topics are selected on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some evidence or suspicion of carcinogenicity. The term 'agent' is used to include individual chemical compounds, groups of related chemical compounds, physical agents (such as radiation) and biological factors (such as viruses). Exposures to mixtures of agents may occur in occupational exposures and as a result of personal and cultural habits (like smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. The IARC information bulletins on agents being tested for carcinogenicity (IARC, 1973–1996) and directories of on-going research in cancer epidemiology (IARC, 1976–1996) often indicate exposures that may be scheduled for future meetings. Ad-hoc working groups convened by IARC in 1984, 1989, 1991, 1993 and

1998 gave recommendations as to which agents should be evaluated in the IARC Monographs series (IARC, 1984, 1989, 1991b, 1993, 1998a,b).

As significant new data on subjects on which monographs have already been prepared become available, re-evaluations are made at subsequent meetings, and revised monographs are published.

4. DATA FOR MONOGRAPHS

The *Monographs* do not necessarily cite all the literature concerning the subject of an evaluation. Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the working groups. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see pp. 25–27). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, unpublished sources of information may be used.

5. THE WORKING GROUP

Reviews and evaluations are formulated by a working group of experts. The tasks of the group are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanism of action; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans.

Working Group participants who contributed to the considerations and evaluations within a particular volume are listed, with their addresses, at the beginning of each publication. Each participant who is a member of a working group serves as an individual scientist and not as a representative of any organization, government or industry. In addition, nominees of national and international agencies and industrial associations may be invited as observers.

6. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, the topics of the monographs are announced and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are

collected by the Carcinogen Identification and Evaluation Unit of IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as MEDLINE and TOXLINE.

For chemicals and some complex mixtures, the major collection of data and the preparation of first drafts of the sections on chemical and physical properties, on analysis, on production and use and on occurrence are carried out under a separate contract funded by the United States National Cancer Institute. Representatives from industrial associations may assist in the preparation of sections on production and use. Information on production and trade is obtained from governmental and trade publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available because their publication could disclose confidential information. Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants, or is used by IARC staff, to prepare sections for the first drafts of monographs. The first drafts are compiled by IARC staff and sent before the meeting to all participants of the Working Group for review.

The Working Group meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, edited and prepared for publication. The aim is to publish monographs within six months of the Working Group meeting.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

7. EXPOSURE DATA

Sections that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are included at the beginning of each monograph.

Most monographs on individual chemicals, groups of chemicals or complex mixtures include sections on chemical and physical data, on analysis, on production and use and on occurrence. In monographs on, for example, physical agents, occupational exposures and cultural habits, other sections may be included, such as: historical perspectives, description of an industry or habit, chemistry of the complex mixture or taxonomy. Mono-

graphs on biological agents have sections on structure and biology, methods of detection, epidemiology of infection and clinical disease other than cancer.

For chemical exposures, the Chemical Abstracts Services Registry Number, the latest Chemical Abstracts primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. For biological agents, taxonomy and structure are described, and the degree of variability is given, when applicable.

Information on chemical and physical properties and, in particular, data relevant to identification, occurrence and biological activity are included. For biological agents, mode of replication, life cycle, target cells, persistence and latency and host response are given. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

The purpose of the section on analysis or detection is to give the reader an overview of current methods, with emphasis on those widely used for regulatory purposes. Methods for monitoring human exposure are also given, when available. No critical evaluation or recommendation of any of the methods is meant or implied. The IARC published a series of volumes, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement* (IARC, 1978–93), that describe validated methods for analysing a wide variety of chemicals and mixtures. For biological agents, methods of detection and exposure assessment are described, including their sensitivity, specificity and reproducibility.

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents which do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of synthesis used in past and present commercial production and different methods of production which may give rise to different impurities are described.

Data on production, international trade and uses are obtained for representative regions, which usually include Europe, Japan and the United States of America. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice, nor does it imply judgement as to their therapeutic efficacy.

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. In the case of mixtures, industries, occupations or processes, information is given about all

agents present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with time and place. For biological agents, the epidemiology of infection is described.

Statements concerning regulations and guidelines (e.g., pesticide registrations, maximal levels permitted in foods, occupational exposure limits) are included for some countries as indications of potential exposures, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccines and therapy, are described.

8. STUDIES OF CANCER IN HUMANS

(*a*) *Types of studies considered*

Three types of epidemiological studies of cancer contribute to the assessment of carcinogenicity in humans — cohort studies, case–control studies and correlation (or ecological) studies. Rarely, results from randomized trials may be available. Case series and case reports of cancer in humans may also be reviewed.

Cohort and case–control studies relate the exposures under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association.

In correlation studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent, mixture or exposure circumstance under study. Because individual exposure is not documented, however, a causal relationship is less easy to infer from correlation studies than from cohort and case–control studies. Case reports generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure. The uncertainties surrounding interpretation of case reports and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, relevant case reports or correlation studies may add materially to the judgement that a causal relationship is present.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed by working groups. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(*b*) *Quality of studies considered*

The Monographs are not intended to summarize all published studies. Those that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when they provide the only data available. Their inclusion does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of the study description.

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. By 'bias' is meant the operation of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between disease and an agent, mixture or exposure circumstance. By 'confounding' is meant a situation in which the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. In evaluating the extent to which these factors have been minimized in an individual study, working groups consider a number of aspects of design and analysis as described in the report of the study. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

Firstly, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken account in the study design and analysis of other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may be more appropriate than those with national rates. Internal comparisons of disease frequency among individuals at different levels of exposure should also have been made in the study.

Thirdly, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. The methods used should preferably have been the generally accepted techniques that have been refined since the mid-1970s. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(*c*) *Inferences about mechanism of action*

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure and time since exposure ceased, are reviewed and summarized when available. The analysis of temporal relationships can be useful in formulating models of carcinogenesis. In particular, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although at best they allow only indirect inferences about the mechanism of action. Special attention is given to measurements of biological markers of carcinogen exposure or action, such as DNA or protein adducts, as well as markers of early steps in the carcinogenic process, such as proto-oncogene mutation, when these are incorporated into epidemiological studies focused on cancer incidence or mortality. Such measurements may allow inferences to be made about putative mechanisms of action (IARC, 1991a; Vainio *et al.*, 1992).

(*d*) *Criteria for causality*

After the individual epidemiological studies of cancer have been summarized and the quality assessed, a judgement is made concerning the strength of evidence that the agent, mixture or exposure circumstance in question is carcinogenic for humans. In making its judgement, the Working Group considers several criteria for causality. A strong association (a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that relative risks of small magnitude do not imply lack of causality and may be important if the disease is common. Associations that are replicated in several studies of the same design or using different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in amount of exposure), and results of studies judged to be of high quality are given more weight than those of studies judged to be methodologically less sound. When suspicion of carcinogenicity arises largely from a single study, these data are not combined with those from later studies in any subsequent reassessment of the strength of the evidence.

If the risk of the disease in question increases with the amount of exposure, this is considered to be a strong indication of causality, although absence of a graded response is not necessarily evidence against a causal relationship. Demonstration of a decline in

risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Although a carcinogen may act upon more than one target, the specificity of an association (an increased occurrence of cancer at one anatomical site or of one morphological type) adds plausibility to a causal relationship, particularly when excess cancer occurrence is limited to one morphological type within the same organ.

Although rarely available, results from randomized trials showing different rates among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, the judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first of all that the studies giving rise to it meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should be consistent with a relative risk of unity for any observed level of exposure and, when considered together, should provide a pooled estimate of relative risk which is at or near unity and has a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency for the relative risk of cancer to increase with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained in this way from several epidemiological studies can apply only to the type(s) of cancer studied and to dose levels and intervals between first exposure and observation of disease that are the same as or less than those observed in all the studies. Experience with human cancer indicates that, in some cases, the period from first exposure to the development of clinical cancer is seldom less than 20 years; studies with latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

9. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (aflatoxins, 4-aminobiphenyl, azathioprine, betel quid with tobacco, bischloromethyl ether and chloromethyl methyl ether (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus ultraviolet A radiation, mustard gas, myleran, 2-naphthylamine, nonsteroidal estrogens, estrogen replacement therapy/steroidal estrogens, solar radiation, thiotepa and vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio *et al.*, 1995). Although this association cannot establish that all agents

and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, **in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is *sufficient evidence* (see p. 24) of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans**. The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans (see p. 27) should also be taken into consideration.

The nature and extent of impurities or contaminants present in the chemical or mixture being evaluated are given when available. Animal strain, sex, numbers per group, age at start of treatment and survival are reported.

Other types of studies summarized include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects; studies in which the end-point was not cancer but a defined precancerous lesion; and experiments on the carcinogenicity of known metabolites and derivatives.

For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

An assessment is made as to the relevance to human exposure of samples tested in experimental animals, which may involve consideration of: (i) physical and chemical characteristics, (ii) constituent substances that indicate the presence of a class of substances, (iii) the results of tests for genetic and related effects, including studies on DNA adduct formation, proto-oncogene mutation and expression and suppressor gene inactivation. The relevance of results obtained, for example, with animal viruses analogous to the virus being evaluated in the monograph must also be considered. They may provide biological and mechanistic information relevant to the understanding of the process of carcinogenesis in humans and may strengthen the plausibility of a conclusion that the biological agent under evaluation is carcinogenic in humans.

(*a*) *Qualitative aspects*

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route and schedule of exposure, species, strain, sex, age, duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

As mentioned earlier (p. 11), the *Monographs* are not intended to summarize all published studies. Those studies in experimental animals that are inadequate (e.g., too short a duration, too few animals, poor survival; see below) or are judged irrelevant to

the evaluation are generally omitted. Guidelines for conducting adequate long-term carcinogenicity experiments have been outlined (e.g. Montesano *et al.*, 1986).

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was adequately monitored, particularly in inhalation experiments; (iii) whether the doses and duration of treatment were appropriate and whether the survival of treated animals was similar to that of controls; (iv) whether there were adequate numbers of animals per group; (v) whether animals of each sex were used; (vi) whether animals were allocated randomly to groups; (vii) whether the duration of observation was adequate; and (viii) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, should be taken into account in the evaluation of tumour response.

When benign tumours occur together with and originate from the same cell type in an organ or tissue as malignant tumours in a particular study and appear to represent a stage in the progression to malignancy, it may be valid to combine them in assessing tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent or mixture induces only benign neoplasms that appear to be end-points that do not readily progress to malignancy, it should nevertheless be suspected of being a carcinogen and requires further investigation.

(*b*) *Quantitative aspects*

The probability that tumours will occur may depend on the species, sex, strain and age of the animal, the dose of the carcinogen and the route and length of exposure. Evidence of an increased incidence of neoplasms with increased level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Both DNA damage and increased cell division are important aspects of carcinogenesis, and cell proliferation is a strong determinant of dose–response relationships for some carcinogens (Cohen & Ellwein, 1990). Since many chemicals require metabolic activation before being converted into their reactive intermediates, both metabolic and pharmacokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship, as could saturation of processes such as DNA repair (Hoel *et al.*, 1983; Gart *et al.*, 1986).

(*c*) *Statistical analysis of long-term experiments in animals*

Factors considered by the Working Group include the adequacy of the information given for each treatment group: (i) the number of animals studied and the number examined histologically, (ii) the number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto *et al.*, 1980; Gart *et al.*, 1986). When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumour type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered), in the case where most differences in survival occur before tumours appear; life-table methods, when tumours are visible or when they may be considered 'fatal' because mortality rapidly follows tumour development; and the Mantel-Haenszel test or logistic regression, when occult tumours do not affect the animals' risk of dying but are 'incidental' findings at autopsy.

In practice, classifying tumours as fatal or incidental may be difficult. Several survival-adjusted methods have been developed that do not require this distinction (Gart *et al.*, 1986), although they have not been fully evaluated.

10. OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

In coming to an overall evaluation of carcinogenicity in humans (see pp. 25–27), the Working Group also considers related data. The nature of the information selected for the summary depends on the agent being considered.

For chemicals and complex mixtures of chemicals such as those in some occupational situations or involving cultural habits (e.g. tobacco smoking), the other data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; toxic effects; reproductive and developmental effects; and genetic and related effects.

Concise information is given on absorption, distribution (including placental transfer) and excretion in both humans and experimental animals. Kinetic factors that may affect the dose–response relationship, such as saturation of uptake, protein binding, metabolic activation, detoxification and DNA repair processes, are mentioned. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data on humans and on animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be of particular importance for extrapolation between species. Data are given on acute and chronic toxic effects (other than cancer), such as

organ toxicity, increased cell proliferation, immunotoxicity and endocrine effects. The presence and toxicological significance of cellular receptors is described. Effects on reproduction, teratogenicity, fetotoxicity and embryotoxicity are also summarized briefly.

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio *et al.*, 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests on p. 18. The available data are interpreted critically by phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system *in vitro* affected the test result. These data are given as listings of test systems, data and references. The data on genetic and related effects presented in the *Monographs* are also available in the form of genetic activity profiles (GAP) prepared in collaboration with the United States Environmental Protection Agency (EPA) (see also Waters *et al.*, 1987) using software for personal computers that are Microsoft Windows® compatible. The EPA/IARC GAP software and database may be downloaded free of charge from *www.epa.gov/gapdb*.

Positive results in tests using prokaryotes, lower eukaryotes, plants, insects and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information about the types of genetic effect produced and about the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g., gene mutations and chromosomal aberrations), while others are to a greater or lesser degree associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour-promoting activity and for cell transformation may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. A critical appraisal of these tests has been published (Montesano *et al.*, 1986).

Genetic or other activity detected in experimental mammals and humans is regarded as being of greater relevance than that in other organisms. The demonstration that an agent or mixture can induce gene and chromosomal mutations in whole mammals indicates that it may have carcinogenic activity, although this activity may not be detectably expressed in any or all species. Relative potency in tests for mutagenicity and related effects is not a reliable indicator of carcinogenic potency. Negative results in tests for mutagenicity in selected tissues from animals treated *in vivo* provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence to rule out carcinogenicity of agents or mixtures that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative proliferation, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that

may lead to misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986).

When available, data relevant to mechanisms of carcinogenesis that do not involve structural changes at the level of the gene are also described.

The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is evaluated by the same criteria as are applied to epidemiological studies of cancer.

Structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent are also described.

For biological agents — viruses, bacteria and parasites — other data relevant to carcinogenicity include descriptions of the pathology of infection, molecular biology (integration and expression of viruses, and any genetic alterations seen in human tumours) and other observations, which might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

11. SUMMARY OF DATA REPORTED

In this section, the relevant epidemiological and experimental data are summarized. Only reports, other than in abstract form, that meet the criteria outlined on p. 11 are considered for evaluating carcinogenicity. Inadequate studies are generally not summarized: such studies are usually identified by a square-bracketed comment in the preceding text.

(*a*) *Exposure*

Human exposure to chemicals and complex mixtures is summarized on the basis of elements such as production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are given when available. Exposure to biological agents is described in terms of transmission and prevalence of infection.

(*b*) *Carcinogenicity in humans*

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

(*c*) *Carcinogenicity in experimental animals*

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. If the agent or mixture produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Negative findings are also summarized. Dose–response and other quantitative data may be given when available.

(*d*) *Other data relevant to an evaluation of carcinogenicity and its mechanisms*

Data on biological effects in humans that are of particular relevance are summarized. These may include toxicological, kinetic and metabolic considerations and evidence of DNA binding, persistence of DNA lesions or genetic damage in exposed humans. Toxicological information, such as that on cytotoxicity and regeneration, receptor binding and hormonal and immunological effects, and data on kinetics and metabolism in experimental animals are given when considered relevant to the possible mechanism of the carcinogenic action of the agent. The results of tests for genetic and related effects are summarized for whole mammals, cultured mammalian cells and nonmammalian systems.

When available, comparisons of such data for humans and for animals, and particularly animals that have developed cancer, are described.

Structure–activity relationships are mentioned when relevant.

For the agent, mixture or exposure circumstance being evaluated, the available data on end-points or other phenomena relevant to mechanisms of carcinogenesis from studies in humans, experimental animals and tissue and cell test systems are summarized within one or more of the following descriptive dimensions:

(i) Evidence of genotoxicity (structural changes at the level of the gene): for example, structure–activity considerations, adduct formation, mutagenicity (effect on specific genes), chromosomal mutation/aneuploidy

(ii) Evidence of effects on the expression of relevant genes (functional changes at the intracellular level): for example, alterations to the structure or quantity of the product of a proto-oncogene or tumour-suppressor gene, alterations to metabolic activation/inactivation/DNA repair

(iii) Evidence of relevant effects on cell behaviour (morphological or behavioural changes at the cellular or tissue level): for example, induction of mitogenesis, compensatory cell proliferation, preneoplasia and hyperplasia, survival of premalignant or malignant cells (immortalization, immunosuppression), effects on metastatic potential

(iv) Evidence from dose and time relationships of carcinogenic effects and interactions between agents: for example, early/late stage, as inferred from epidemiological studies; initiation/promotion/progression/malignant conversion, as defined in animal carcinogenicity experiments; toxicokinetics

These dimensions are not mutually exclusive, and an agent may fall within more than one of them. Thus, for example, the action of an agent on the expression of relevant genes could be summarized under both the first and second dimensions, even if it were known with reasonable certainty that those effects resulted from genotoxicity.

12. EVALUATION

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent, mixture or exposure circumstance to a higher or lower category than a strict interpretation of these criteria would indicate.

(*a*) *Degrees of evidence for carcinogenicity in humans and in experimental animals and supporting evidence*

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency) nor to the mechanisms involved. A classification may change as new information becomes available.

An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of degree of evidence.

(i) *Carcinogenicity in humans*

The applicability of an evaluation of the carcinogenicity of a mixture, process, occupation or industry on the basis of evidence from epidemiological studies depends on the variability over time and place of the mixtures, processes, occupations and industries. The Working Group seeks to identify the specific exposure, process or activity which is considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

Evidence suggesting lack of carcinogenicity: There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to

the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure. A conclusion of 'evidence suggesting lack of carcinogenicity' is inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

(ii) *Carcinogenicity in experimental animals*

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Exceptionally, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset.

Limited evidence of carcinogenicity: The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

Inadequate evidence of carcinogenicity: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

Evidence suggesting lack of carcinogenicity: Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent or mixture is not carcinogenic. A conclusion of evidence suggesting lack of carcinogenicity is inevitably limited to the species, tumour sites and levels of exposure studied.

(*b*) *Other data relevant to the evaluation of carcinogenicity and its mechanisms*

Other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is then described. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and pharmacokinetics, physicochemical parameters and analogous biological agents.

Data relevant to mechanisms of the carcinogenic action are also evaluated. The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is assessed, using terms such as weak, moderate or strong. Then, the Working Group assesses if that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans come from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(*c*) *Overall evaluation*

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity to humans of an agent, mixture or circumstance of exposure.

An evaluation may be made for a group of chemical compounds that have been evaluated by the Working Group. In addition, when supporting data indicate that other, related compounds for which there is no direct evidence of capacity to induce cancer in humans or in animals may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of compounds if the strength of the evidence warrants it.

The agent, mixture or exposure circumstance is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent, mixture or exposure circumstance is a matter of scientific judgement, reflecting the strength of the evidence derived from studies in humans and in experimental animals and from other relevant data.

Group 1 — The agent (mixture) is carcinogenic to humans.
The exposure circumstance entails exposures that are carcinogenic to humans.

This category is used when there is *sufficient evidence* of carcinogenicity in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is *sufficient evidence* of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures and exposure circumstances are assigned to either group 2A (probably carcinogenic to humans) or group 2B (possibly carcinogenic to humans) on the basis of epidemiological and experimental evidence of carcinogenicity and other relevant data.

Group 2A — The agent (mixture) is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to humans.

This category is used when there is *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals. In some cases, an agent (mixture) may be classified in this category when there is *inadequate evidence* of carcinogenicity in humans, *sufficient evidence* of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture or exposure circumstance may be classified in this category solely on the basis of *limited evidence* of carcinogenicity in humans.

Group 2B — The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans.

This category is used for agents, mixtures and exposure circumstances for which there is *limited evidence* of carcinogenicity in humans and less than *sufficient evidence* of carcinogenicity in experimental animals. It may also be used when there is *inadequate evidence* of carcinogenicity in humans but there is *sufficient evidence* of carcinogenicity in experimental animals. In some instances, an agent, mixture or exposure circumstance for which there is *inadequate evidence* of carcinogenicity in humans but *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

Group 3 — The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents, mixtures and exposure circumstances for which the *evidence of carcinogenicity* is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents (mixtures) for which the *evidence of carcinogenicity* is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category

when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category.

Group 4 — The agent (mixture) is probably not carcinogenic to humans.

This category is used for agents or mixtures for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents or mixtures for which there is *inadequate evidence* of carcinogenicity in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group.

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GENERAL REMARKS ON THE SUBSTANCES CONSIDERED

This eighty-sixth volume of *IARC Monographs* considers cobalt (with or without tungsten carbide) in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide.

Most of the materials evaluated in this volume are poorly soluble solid materials that are deposited in particulate form in the lung, where they may be retained for long periods of time. In this respect, they should be considered as 'particulate toxicants', the toxic effects of which are regulated not only by their chemical composition but also by their particle size and surface properties.

Workers in the hard-metal industry can have significant exposures to metallic cobalt particles in general in the presence but occasionally in the absence of tungsten carbide. Cobalt and cobalt compounds were evaluated in volume 52 (1991) as being *possibly carcinogenic to humans* (*Group 2B*), and the evidence of carcinogenicity in humans was *inadequate*. Since that time, new epidemiological studies of the hard-metal industry have been conducted in Sweden and in France and are evaluated here. Exposure to metallic cobalt is also prevalent in the cobalt production industry, and studies on that industry were also considered in the evaluation of cobalt. Because most data from the hard-metal industry deal with mixtures of cobalt and tungsten carbide, the Working Group also evaluated studies of tungsten miners, especially in China. Although these studies explored an association between exposure to silica and lung cancer and no data on exposure to tungsten were available, risks for lung cancer were nevertheless presented separately for tungsten miners. These were not increased compared with the reference population, but there is major potential for confounding by silica and other carcinogens in these studies.

No new studies in experimental animals were available for cobalt compounds used in the hard-metal industry. Nevertheless, this volume re-evaluates some of the experimental evidence for cobalt that was presented in the previous volume. The Working Group questioned the relevance of the routes of administration used in some of the animal carcinogenesis bioassays for the evaluation of carcinogenicity of cobalt metal and cobalt alloys. These included, for example, intramuscular injection into rats of cobalt metal powder or cobalt–chromium–molybdenum alloy, which produced sarcomas at the site of injection. The bioassays were reviewed again in this volume and the Working Group maintained the same conclusion as that reached in the previous monograph.

The semiconductor industry is a rapidly growing and changing industry that uses several compounds which have been evaluated as being potentially carcinogenic to humans. Inhalation studies by the National Toxicology Program have recently become available on two metal compounds used in this industry — gallium arsenide and indium phosphide. The available human epidemiological evidence from studies of the semiconductor industry is summarized and evaluated, although this is not extensive and is not particularly informative for the monographs on gallium arsenide and indium phosphide. Exposures to gallium arsenide and indium phosphide in the semiconductor industry may be very low, and other potential carcinogens present in this industry include trichloroethylene (*Group 2A*; IARC, 1995) and ultraviolet radiation (*Group 2A*; IARC, 1992).

In addition, there have been indications of adverse reproductive and developmental effects in workers in the semiconductor industry, although it has been suggested that these may be attributed in part to factors that are unrelated to employment in this industry. Therefore, more comprehensive epidemiological investigations of the semiconductor industry are needed.

Although they are not used in either the hard-metal or semiconductor industries, inhalation studies by the National Toxicology Program have recently become available on cobalt sulfate heptahydrate and vanadium pentoxide. Because the Working Group that convened to elaborate this volume had considerable expertise in metal carcinogenicity, it was considered advantageous to evaluate these compounds also. The evaluation of cobalt sulfate heptahydrate in this volume brings up to date the evaluations of cobalt compounds that appear in volume 52.

THE MONOGRAPHS

METALLIC COBALT PARTICLES
(WITH OR WITHOUT TUNGSTEN CARBIDE)

METALLIC COBALT PARTICLES (WITH OR WITHOUT TUNGSTEN CARBIDE)

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Metallic cobalt

Chem. Abstr. Serv. Reg. No.: 7440-48-4
Deleted CAS Reg. No.: 177256-35-8; 184637-91-0; 195161-79-6
Chem. Abstr. Name: Cobalt
IUPAC Systematic Name: Cobalt
Synonyms: C.I. 77320; Cobalt element; Cobalt-59

Cobalt sulfate heptahydrate

Chem. Abstr. Serv. Reg. No.: 10026-24-1
Chem. Abstr. Name: Sulfuric acid, cobalt(2+) salt (1:1), heptahydrate
IUPAC Systematic Name: Cobaltous sulfate heptahydrate
Synonyms: Cobalt monosulfate heptahydrate; cobalt(II) sulfate heptahydrate; cobalt(II) sulfate (1:1), heptahydrate

Tungsten carbide

Chem. Abstr. Serv. Reg. No.: 12070-12-1
Deleted CAS Reg. No.: 52555-87-0; 182169-08-0; 182169-11-5; 188300-42-7; 188300-43-8; 188300-44-9; 188300-45-0
Chem. Abstr. Name: Tungsten carbide
IUPAC Systematic Name: Tungsten carbide
Synonyms: Tungsten carbide (WC); tungsten monocarbide; tungsten monocarbide (WC)

1.1.2 *Molecular formulae and relative molecular mass*

Co	Relative atomic mass: 58.93
$CoSO_4.7H_2O$	Relative molecular mass: 281.10
WC	Relative molecular mass: 195.85

1.1.3 *Chemical and physical properties of the pure substance* (from Lide, 2003, unless otherwise specified)

Cobalt

(*a*) *Description*: Hexagonal or cubic crystalline grey metal; exists in two allotropic modifications; both forms can exist at room temperature, although the hexagonal form is more stable than the cubic form (O'Neil, 2001)
(*b*) *Boiling-point*: 2927 °C
(*c*) *Melting-point*: 1495 °C
(*d*) *Density*: 8.86 g/cm^3
(*e*) *Solubility*: Soluble in dilute acids; ultrafine metal cobalt powder is soluble in water at 1.1 mg/L (Kyono *et al.*, 1992)

Cobalt sulfate heptahydrate

(*a*) *Description*: Pink to red monoclinic, prismatic crystals (O'Neil, 2001)
(*b*) *Melting-point*: 41 °C, decomposes
(*c*) *Density*: 2.03 g/cm^3
(*d*) *Solubility*: Soluble in water; slightly soluble in ethanol and methanol (O'Neil, 2001)

Tungsten carbide

(*a*) *Description*: Grey hexagonal crystal
(*b*) *Boiling-point*: 6000 °C (Reade Advanced Materials, 1997)
(*c*) *Melting-point*: 2785 °C
(*d*) *Density*: 15.6 g/cm^3
(*e*) *Solubility*: Insoluble in water; soluble in nitric and hydrofluoric acids

1.1.4 *Technical products and impurities*

Cobalt-metal and tungsten carbide powders are produced widely in high purity for use in the hard-metal industry, in the manufacture of superalloys and for other applications. [Superalloys are alloys usually based on group VIIIA elements (iron, cobalt, nickel) developed for elevated temperature use, where relatively severe mechanical stressing is encountered and where high surface stability is frequently required (Cobalt Development Institute, 2003).] Specifications of cobalt-metal powders are closely controlled to meet the requirements of particular applications. Commercial cobalt-metal powders are available

in purities ranging from 99% to ≥ 99.999% in many grades, particle size ranges and forms; commercial tungsten carbide powders are available in purities ranging from 93% to 99.9%, also in many grades, particle size ranges and forms. Tables 1 and 2 show the specifications for selected cobalt-metal and tungsten-carbide powder products.

1.1.5 *Analysis*

(*a*) *Biological monitoring*

The presence of cobalt in samples of whole blood, plasma, serum and urine is used as a biological indicator of exposure to cobalt (Ichikawa *et al.*, 1985; Ferioli *et al.*, 1987; Angerer *et al.*, 1989). Soluble cobalt compounds are readily absorbed and excreted in the urine (see Section 4.1) and therefore urinary cobalt is considered a good indicator of exposure to these, but not to insoluble cobalt compounds (Cornelis *et al.*, 1995).

For an accurate determination of cobalt concentration in body fluids, it is necessary to use blood collection devices which do not themselves produce detectable amounts of cobalt. All containers must be washed with high purity acids. Urine samples may be acidified with high purity nitric acid and stored at 4 °C for one week, or at –20 °C for longer periods, prior to analysis (Minoia *et al.*, 1992; Cornelis *et al.*, 1995).

(*b*) *Analytical methods for workplace air and biological monitoring*

Analytical methods used until 1988 for the determination of cobalt in air particulates (for workplace air monitoring) and in biological materials (for biological monitoring) have been reviewed in a previous monograph on cobalt and its compounds (IARC, 1991). These methods are primarily flame and graphite-furnace atomic absorption spectrometry (F-AAS, and GF-AAS, respectively) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Minor applications of electrochemical methods, namely adsorption voltametry, differential pulse anodic stripping voltametry and neutron activation analysis (NAA) for the determination of cobalt in serum have also been mentioned (IARC, 1991; Cornelis *et al.*, 1995).

Inductively coupled plasma mass spectrometry (ICP-MS) has become more widely available since the early 1990s, and is increasingly used for multi-elemental analysis of human blood, serum or urine, including determination of cobalt concentrations in these body fluids (Schmit *et al.*, 1991; Moens & Dams, 1995; Barany *et al.*, 1997; Sariego Muñiz *et al.*, 1999, 2001).

(*c*) *Reference values for occupationally non-exposed populations*

Normal concentrations of cobalt in the body fluids of healthy individuals are uncertain. Cornelis *et al.* (1995) give a range of 0.1–1 µg/L for cobalt concentrations in urine. Results obtained in national surveys of healthy adults yielded a mean cobalt concentration in urine of 0.57 µg/L in a population sample in Italy (Minoia *et al.*, 1990) and of 0.46 µg/L in a population sample in the United Kingdom of Great Britain and Northern

Table 1. Specifications for selected technical cobalt-metal powder products

Minimum % cobalt	Maximum %[a] contaminants permitted	Grade/particle size/crystal structure	Country of production	Reference
99.85	C, 0.02; S, 0.001; P, 0.01; Fe, 0.015	Not stated	India	Jayesh Group (2003)
> 99.95	C, 0.0015–0.002; Cu, < 0.0005; H, < 0.0005; Fe, < 0.001; Pb, < 0.0002; Ni, 0.03–0.05; N, < 0.0001; O, < 0.005; Si, < 0.0003; S, 0.0002–0.035; Zn, 0.0001–0.0002	Electrolytic and S-type/ 25 mm cut squares	Canada	Falconbridge (2002)
99.9	Bi, < 0.00002; C, 0.0025; Cu, 0.0001; H, 0.0002; Fe, 0.0004; Pb, 0.0003; Ni, 0.095; N, 0.0004; O, 0.005; Se, < 0.00002; S, 0.0005; Zn, 0.0008	Electrolytic rounds/button-shaped pieces circa 35 mm in diameter and circa 5 mm thick	Canada	Inco Ltd (2003)
99.999	[mg/kg] Cu, Cd, Pb, Cr, Al, Ag, Na, Sb, W, Li, Mg, Mn, Mo, Si, Ti, Cl, K, Ca and Ni, < 1; Fe, < 2; Zn and As, < 5; S, < 10; C, < 20	Shiny silver-grey cathode plates/hexagonal	Belgium	Umicore Specialty Metals (2002)
99.5	Ni, 0.05; Fe, 0.11; Mn, 0.01; Cu, 0.007; Pb, < 0.001; Zn, 0.003; Si and Ca, 0.04; Mg, 0.02; Na, 0.005; S, 0.01; C, 0.025; O_2, 0.30	Coarse particle/400 or 100 mesh/50% hexagonal, 50% cubic	Belgium	Umicore Specialty Metals (2002)
99.8	Ni, 0.15; Ag, 0.02; Fe, 0.003; Mg, Mn and Cu, < 0.0005; Zn and Na, 0.001; Al, Ca and Si, < 0.001; Pb, < 0.002; S, 0.006; C, 0.07; O_2, 0.5	5M powder/3.3–4.7 µm/ 90% hexagonal, 10% cubic	Belgium	Umicore Specialty Metals (2002)

Table 1 (contd)

Minimum % cobalt	Maximum %[a] contaminants permitted	Grade/particle size/crystal structure	Country of production	Reference
99.88	Ni, 0.05; Fe, 0.005; Mg, Mn, Pb and S, < 0.001; Ca, Cu and Zn, 0.003; Si, < 0.002; Na 0.002; C, 0.015; O_2, 0.35	Extra fine powder/1.2–1.5 µm/70% hexagonal, 30% cubic	Belgium	Umicore Specialty Metals (2002)
99.8	Ni, 0.10; Ag, 0.12; Al, Fe, Na and Pb, < 0.001; Cu, Mg and Mn, < 0.0005; Zn, 0.0011; Ca, 0.0013; Si, < 0.003; S, 0.005; C, 0.22; O_2, 0.8	Half micron powder/0.55 µm/80% hexagonal, 20% cubic	Belgium	Umicore Specialty Metals (2002)
99.7	[mg/kg] C, 1000; Ni and Cl, 500; Fe and Ca, 70; Na, 60; Mg, 30; Cu and Zn, 20; Al, Mn, Pb and S, < 10; Si, < 20; O_2, 0.8%	Submicron-size powder/0.8 µm/85% hexagonal, 15% cubic	Belgium	Umicore Specialty Metals (2002)
99.8	Ni, 0.15; Ag, 0.12; Fe and Na, 0.001; Al, Cu, Mg and Mn, < 0.0005; Zn, 0.0013; Ca, 0.0015; Pb, < 0.002; Si, < 0.001; S, 0.006; C, 0.18; O_2, 0.7	Ultrafine powder/0.9 µm/90% hexagonal, 10% cubic	Belgium	Umicore Specialty Metals (2002)
> 99.8	[mg/kg] Ca, Fe and Si, < 100; Ni, < 400–1000; O_2, < 0.8%	Extrafine powder/1.05–1.45 µm	France	Eurotungstene Metal Powders (2003)
99.80	C and Ni, 0.20; Ag, 0.15; Fe, 0.02; Cu, 0.005; S, 0.01; O, 0.80	Ultrafine powder/0.9–8.0 µm	Luxembourg	Foxmet SA (2003)
99.8	[mg/kg] Ni, 600; C, 300; Fe, 100; Cu and S, 50; O, 0.50%	Extrafine powder/1.40–3.90 µm	Luxembourg	Foxmet SA (2003)

Table 1 (contd)

Minimum % cobalt	Maximum %[a] contaminants permitted	Grade/particle size/crystal structure	Country of production	Reference
99.20	[mg/kg] Ni and Fe, 1000; Ca, 750; C and S, 300; O, 0.50%	Fine powder-400 mesh/4.2–14.0 µm	Luxembourg	Foxmet SA (2003)
99.90	Ni, 0.30; C, 0.10; Fe and S, 0.01; Cu, 0.001; O, 0.60	Fine powder-5M/4.0 µm	Luxembourg	Foxmet SA (2003)
99.80	Ni, 0.05; C, 0.10; Fe, 0.003; S, 0.03; Cu, 0.002	Coarse powder-'S' grade/75–600 µm	Luxembourg	Foxmet SA (2003)
99.8	[mg/kg] C, 1000; S, 350; Ni, 200; Fe, 35; Cu and Zn, 15	Coarse powder-'DGC' grade/45–600 µm	Luxembourg	Foxmet SA (2003)
Not stated	Not stated	Coarse powder-100 & 400 mesh; battery grade briquette; extrafine powder (standard & high density); submicron (0.8 µm) powder	USA	OM Group (2003)

[a] Unless stated otherwise

Table 2. Specifications for selected technical tungsten-carbide (WC) powder products

Minimum % WC	Maximum %[a] contaminants permitted	Grade/particle size	Country of production	Reference
Not stated	Total C, 6.11–6.16; free C, 0.03; [mg/kg] Al, Cr and Na, 10; Ca and Ni, 20; Co, Cu, K, Mg and Mn, 5; Mo, 50; Si and Fe, 30	100–200 mesh 0.7–20.0 µm	Israel	Metal-Tech Ltd (2003)
93–94	Total C, 6; free C, 0.04	Mesh size, 200	India	Jayesh Group (2003)
99.70–99.90	Total C, 6.08–6.29; free C, 0.05–0.16; Fe, 0.02; Mo, 0.01	Standard grade/0.7–12 µm	Japan	Japan New Metals Co. Ltd (2003)
Not stated	Total C, 6.05–6.25; free C, 0.10; Fe, 0.05; Mo, 0.02; Cr, 1; V, 1	Fine grade/0.45–0.75 µm	Japan	Japan New Metals Co. Ltd (2003)
99.8	Total C, 6.13; free C, 0.10; Fe, 0.05; Mo, 0.02	Standard grade/0.7–7.1 µm	Japan	A.L.M.T. Corp. (2003)
99.8	Total C, 6.13; free C, 0.05; Fe, 0.02; Mo, 0.02	Coarse grade/2.5–16 µm	Japan	A.L.M.T. Corp. (2003)
Not stated	Total C, 6.15–6.20; free C, 0.15–0.25; Fe, 0.02; Mo, 0.02	Ultrafine grade/0.1–0.70 µm	Japan	A.L.M.T. Corp. (2003)
Not stated	Total C, 6.11–6.18; free C, < 0.08; [mg/kg] Al and Ca, < 10; Cr, < 40; Fe, < 200; K, Mg and Na, < 15; Mo, < 50; Ni, < 25; Si, < 40; V, 1400–2000; O_2, < 0.16–0.25%	0.6–1.1 µm (doped with 0.2% VC)	France	Eurotungstene Metal Powders (2003)
Not stated	Combined C, 6.05 min.; free C, 0.08; O_2, 0.025–0.030	2.6–5.5 µm	France	Eurotungstene Metal Powders (2003)

Table 2 (contd)

Minimum % WC	Maximum %[a] contaminants permitted	Grade/particle size	Country of production	Reference
Not stated	Total C, 3.9–4.2; free C, 0.1; Fe, 0.4	Fused powders (eutectic mixture of WC and W_2C)/ < 45–450 µm	France	Eurotungstene Metal Powders (2003)
Not stated	Not stated	DS/0.45–2.5 µm MAS/5.0–50 µm HC/2.5–14 µm DR/3–10 µm MA/4–12 µm	Germany	Starck (2003)
99.7	Total C, 6.13; free C, 0.06; [mg/kg] Fe and Mo, 250; Co, 100; Cr, 75; Ca, Ni and Si, 50; Al, 25; Na, 20; Cu, 15	Fine grade powder/0.9–6.3 µm	Luxembourg	Foxmet SA (2003)
Not stated	Total C, 3.90–4.20 ; free C, 0.10; Fe, 0.40; O, 0.10	Fused powder/0–150 µm	Luxembourg	Foxmet SA (2003)
80–88% WC & 12–20% Co	Not stated	pre-alloyed WC/Co powder/ 0–300 µm	Luxembourg	Foxmet SA (2003)
10–50% WC & 50–90% Co	Not stated	Ready-mixed powder	Luxembourg	Foxmet SA (2003)
Not stated	Total C, 6.08–6.24; free C, 0.05; Fe, 0.03; Mo and Nb, 0.15; Ta, 0.1; Ti, 0.20	Macrocrystalline powder/0–420 µm	USA	Kennametal (2003)

Table 2 (contd)

Minimum % WC	Maximum %[a] contaminants permitted	Grade/particle size	Country of production	Reference
Not stated	Not stated	Conventional carburized powder/0.8–4.8 µm Cast carbide vacuum-fused powder/44–2000 µm Chill cast carbide/37–420 µm) Sintered WC/Co hard metal/44–2000 µm	USA	Kennametal (2003)

[a] Unless stated otherwise

Ireland (White & Sabbioni, 1998). Significant differences between concentrations of cobalt in the urine of men and women (median values of [0.22] and [0.39 µg/L], respectively) were reported by Kristiansen *et al.* (1997).

Concentrations of cobalt in blood and serum are expected to be at the lower end of the 0.1–1 µg/L range (Versieck & Cornelis, 1980); a median cobalt concentration in serum of 0.29 µg/L was determined by Iyengar and Woittiez (1988). In an Italian population, Minoia *et al.* (1990) reported median concentrations of cobalt in blood and serum of 0.39 µg/L and 0.21 µg/L, respectively. Alimonti *et al.* (2000) recently reported cobalt concentrations in the range of 0.20–0.43 µg/L in the serum of newborns from an urban area of Rome, suggesting that there is no age dependence in serum cobalt concentrations.

1.2 Production and use

1.2.1 *Production*

(*a*) *Cobalt*

World production of refined cobalt has increased steadily over the last decade, due partly to new operations and partly to a net increase in production by established producers. World demand for cobalt is strongly influenced by general economic conditions and by demand from industries that consume it in large quantities, such as superalloy melters and manufacturers of rechargeable batteries (Shedd, 2003).

World cobalt resources identified to date are estimated at about 15 million tonnes. The vast majority of these resources are in nickel-bearing laterite deposits or, to a much smaller extent, in nickel–copper sulfide deposits in Australia, Canada and the Russian Federation and in the sedimentary copper deposits of the Democratic Republic of Congo and Zambia. In addition, it is postulated that millions of tonnes of cobalt exist in manganese nodules and crusts on the ocean floor (Shedd, 2003).

Cobalt is extracted from several mineral ores, including arsenide, sulfoarsenide (cobaltite), sulfide (chalcocite, carrollite), arsenic-free cobalt–copper (heterogenite), lateritic and oxide ores. Cobalt is recovered from concentrates and occasionally directly from the ore itself by hydrometallurgical, pyrometallurgical and electrometallurgical processes. Cobalt powder can be produced by a number of methods, but those of industrial importance involve the reduction of oxides, the pyrolysis of carboxylates, and the reduction of cobalt ions in aqueous solution with hydrogen under pressure. Very pure cobalt powder is prepared by the decomposition of cobalt carbonyls. Grey cobalt(II) oxide (CoO) or black cobalt(II)/cobalt(III) oxide (Co_3O_4) is reduced to the metal powder by carbon monoxide or hydrogen. The purity of the powder obtained is 99.5% with a particle size of approximately 4 µm, although the density and particle size of the final product depend on the reduction conditions and on the particle size of the parent oxide. The thermal decomposition of cobalt carboxylates such as formate and oxalate in a controlled reducing or neutral atmosphere produces a high-purity (about 99.9%), light, malleable cobalt powder, with a particle size of approximately 1 µm which is particularly suitable for the manufacture of hard metals

(see below). The particle size, form and porosity of the powder grains can be changed by altering the pyrolysis conditions (Hodge, 1993; Donaldson, 2003).

World mine and refinery production figures for cobalt from 1997 to 2001 are presented in Tables 3 and 4, respectively (Shedd, 2001). Available information indicates that cobalt is manufactured by five companies in China, four companies each in India and the United States of America (USA), three companies in Japan, and two companies each in Belgium, Brazil, Canada, the Netherlands, the Russian Federation and the United Kingdom. Argentina, France, Germany, Italy, Mexico, Norway, the Philippines, Poland and Turkey each have one manufacturing company (Chemical Information Services, 2003). Other important cobalt-manufacturing countries include Australia, the Democratic Republic of Congo, Finland, Morocco and Zambia (Shedd, 2001).

Table 3. World cobalt mine production by country (in tonnes of cobalt)[a]

Country[b]	1997	1998	1999	2000	2001
Australia	3 000	3 300	4 100	5 600	6 200
Botswana	334	335	331	308	325
Brazil	400	400	700	900	1 100
Canada	5 709	5 861	5 323	5 298	5 334
China	200	40	250	90	150
Cuba	2 358	2 665	2 537	2 943	3 411
Democratic Republic of the Congo	3 500	5 000	6 000	7 000	4 700
France (New Caledonia)	1 000	1 000	1 100	1 200	1 400
Kazakhstan	300	300	300	300	300
Morocco	714	287	863	1 305	1 300
Russian Federation	3 300	3 200	3 300	3 600	3 800
South Africa	465	435	450	580	550
Zambia	6 037	11 900	5 640	4 600	8 000
Zimbabwe	126	138	121	79	95
Total	27 400	34 900	31 000	33 800	36 700

From Shedd (2001)

[a] Figures represent recoverable cobalt content of ores, concentrates or intermediate products from copper, nickel, platinum or zinc operations.

[b] In addition to the countries listed, Bulgaria, Indonesia, the Philippines and Poland are known to produce ores that contain cobalt, but information is inadequate for reliable estimates of output levels.

(*b*) *Metallic carbides*

Carbon reacts with most elements of the periodic table to form a diverse group of compounds known as carbides, some of which have extremely important technological applications.

Table 4. World cobalt refinery production by country (in tonnes of cobalt)[a]

Country[b]	Product	1997	1998	1999	2000	2001
Australia	Metal (including metal powder), oxide, hydroxide	617	1 395	1 700	2 610	3 470
Belgium	Metal powder, oxide, hydroxide	1 200	1 200	950	1 110	1 090
Brazil	Metal	266	364	651	792	889
Canada	Metal (including metal powder), oxide	3 792	4 415	4 196	4 364	4 378
China	Metal	470	410	300	410	450
Democratic Republic of the Congo	Metal	2 808	4 490	5 180	4 320	4 071
Finland	Metal, powder, salts	5 000	5 250	6 200	7 700	8 100
France	Chloride	159	172	181	204	199
India	Metal, salts	110	120	120	206	250
Japan	Metal	264	329	247	311	350
Morocco	Metal	225	242	472	1 200	1 200
Norway	Metal	3 417	3 851	4 009	3 433	3 314
Russian Federation	Unspecified	4 100	3 500	3 600	4 400	5 000
South Africa	Metal, powder, sulfate	316	296	306	397	371
Uganda	Metal	0	0	77	420	634
Zambia	Metal	4 403	4 837	4 236	3 342	4 657
Total		27 100	30 900	32 400	35 200	38 400

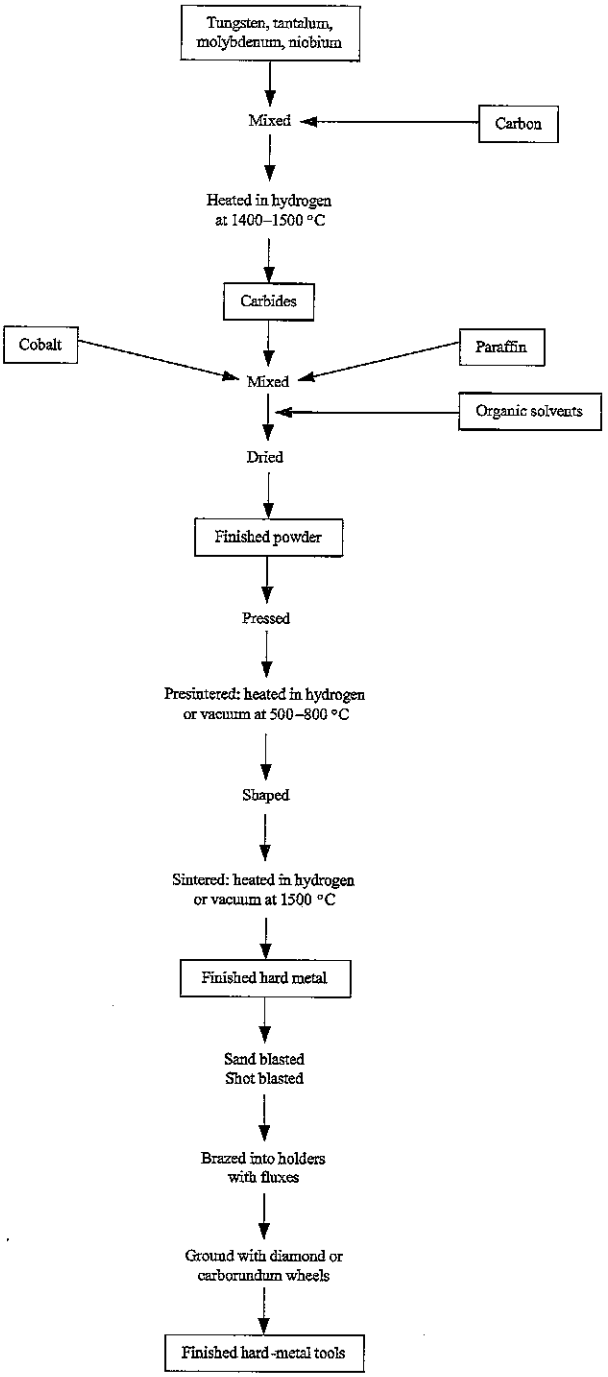
From Shedd (2001)

[a] Figures represent cobalt refined from ores, concentrates or intermediate products and do not include production of downstream products from refined cobalt.

[b] In addition to the countries listed, Germany and Slovakia may produce cobalt, but available information is inadequate to make reliable estimates of production.

Metallic carbides (industrial hard carbides) comprise the carbides of metals of groups IVB–VIB. Metallic carbides combine the physical properties of ceramics with the electronic nature of metals; they are hard and strong, but at the same time good conductors of heat and electricity (Oyama & Kieffer, 1992). Tungsten carbide, titanium carbide and tantalum carbide are used as structural materials in extremely high temperatures or in corrosive atmospheres. Carbides are generally stable at high temperatures and metallic carbides are prepared by the direct reaction between carbon and metals at high temperatures. For example, fine tungsten powders blended with carbon and heated in a hydrogen atmosphere at 1400–1500 °C produce tungsten carbide (WC) particles varying in size from 0.5 to 30 μm. Each particle is composed of numerous tungsten carbide crystals. Small amounts of vanadium, chromium or tantalum are sometimes added to tungsten and carbon powders before carburization to produce very fine (< 1 μm) tungsten carbide powders (Stoll & Santhanam, 1992) (Figure 1).

Figure 1. Steps in the manufacture of hard-metal tools



From Kusaka *et al.* (1986)

Available information indicates that tungsten carbide is manufactured by five companies in the USA, four companies in Japan, three companies in Germany and two companies each in Brazil and France. Argentina, Austria, Canada, India, Israel, Portugal and the Republic of Korea each have one manufacturing company (Chemical Information Services, 2003).

(c) *Hard metals*

Hard metals are materials in which metallic carbides are bound together or cemented by a soft and ductile metal binder, usually cobalt or nickel. Although the term 'cemented carbide' is widely used in the USA, these materials are better known internationally as 'hard metals' (Santhanam, 1992). Hard metals are manufactured by a powder metallurgy process consisting of a sequence of carefully-controlled steps designed to obtain a final product with specific properties, microstructure and performance (Santhanam, 1992).

Figure 1 illustrates the steps involved in the preparation of hard metals and the manufacture of hard-metal tools. The carbides or carbide solid solution powders are prepared, blended, compacted, presintered and shaped, and subjected to sintering and postsintering operations. The sintered product (finished hard metal) may be either put to use directly, or ground, polished and coated (Santhanam, 1992). [Sintering is the agglomeration of metal powders at temperatures below their melting-point, as in powder metallurgy; while heat and pressure are essential, decrease in surface area is the critical factor; sintering increases strength, conductivity and density (Lewis, 2001).]

The binder metal (cobalt or nickel) is obtained as a very fine powder and is blended with carbide powders in ball mills, vibratory mills or attritors [grinding machines] using carbide balls. The mills are lined with carbide, low-carbon steel or stainless-steel sleeves. Intensive milling is necessary to break up the initial carbide crystallites and disperse the cobalt among the carbide particles to enhance wetting by cobalt during sintering. Milling is performed under an organic liquid such as alcohol, hexane, heptane or acetone; in the process, a solid lubricant such as paraffin wax or poly(ethylene glycol) is added to the powder blend to strengthen the pressed or consolidated powder mix. After milling, the organic liquid is removed by drying. In a spray-drying process, commonly used in the hard-metal industry, a hot inert gas such as nitrogen impinges on a stream of carbide particles to produce free-flowing spherical powder aggregates (Santhanam, 1992).

The milled and dried grade powders are pressed to desired shapes in hydraulic or mechanical presses. Special shapes may require a presintering operation followed by machining or grinding to the final form. Cold isostatic pressing, followed by green forming [forming the powder into the desired shape], is also common in the manufacture of wear-resistant components and metal-forming tools. Rods and wires are formed by an extrusion process (Santhanam, 1992).

For sintering, the pressed compacts are set on graphite trays and are heated initially to approximately 500 °C in an atmosphere of hydrogen or in a vacuum to remove the lubricant. Subsequently, the compacts are heated under vacuum to a final sintering temperature ranging from 1350 to 1550 °C, depending on the amount of metal binder and the micro-

structure desired. During final sintering, the binder melts and draws the carbide particles together, shrinking the compact by 17–25% (on a linear scale) and yielding a virtually pore-free, fully dense product (Santhanam, 1992).

In the 1970s, the hard-metal industry adapted hot isostatic pressing (HIP) technology to remove any residual internal porosity, pits or flaws from the sintered product. The HIP process involves reheating vacuum-sintered material to a temperature 25–50 °C less than the sintering temperature under a gaseous (argon) pressure of 100–150 MPa (14 500–21 750 psi). An alternative method developed in the early 1980s, the sinter-HIP process, uses low-pressure HIP, up to 7 MPa (1015 psi), combined with vacuum sintering. The pressure is applied at the sintering temperature when the metallic binder is still molten, resulting in void-free products.

After sintering, hard-metal products that require shaping to meet surface finish, tolerance or geometric requirements undergo grinding with metal-bonded diamond wheels or lapping with diamond-containing slurries (Santhanam, 1992).

Recycling of hard-metal scrap is of growing importance and several methods are available. In one method, the scrap is heated to 1700–1800 °C in a vacuum furnace to vaporize some of the cobalt and embrittle the material. After removal from the furnace, the material is crushed and screened. [Screening is the separation of an aggregate mixture into two or more portions according to particle size, by passing the mixture through one or more standard screens.] In chemical recycling, the cobalt is removed by leaching, leaving carbide particles intact. In the zinc reclaim process, commercialized in the late 1970s, the cleaned scrap is heated with molten zinc in an electric furnace at approximately 800 °C under an inert gas. The zinc reacts with the cobalt binder and the carbide pieces swell to more than twice their original volume. The zinc is distilled off under vacuum and reclaimed. The carbide pieces are pulverized and screened to produce a fine powder. The cobalt is still present in the particles and there is no change in grain size from the original sintered scrap. The coldstream reclaim method uses a high velocity airstream to accelerate hard-metal particles with sufficient energy to cause them to fracture against a target surface. This process, so called because the air cools as it expands from the nozzles, is used in combination with the zinc reclaim process (Santhanam, 1992).

(*d*) *Cobalt alloys*

Multimetallic complexes, which include cobalt alloys, are the components in tool steels and Stellite-type alloys that are responsible for hardness, wear resistance and excellent cutting performance (Oyama & Kieffer, 1992). [Stellite is an alloy containing cobalt and chromium, and sometimes other metals.]

1.2.2 *Use*

Cobalt compounds have been used as blue colouring agents in ceramic and glass for thousands of years, although most of the blue colour of ancient glasses and glazes has been found to be due to copper. Cobalt has been found in Egyptian pottery dating from

about 2600 BC, in Persian glass beads dating from 2250 BC, in Greek vases and in pottery from Persia and Syria from the early Christian era, in Chinese pottery from the Tang (600–900 AD) and Ming (1350–1650 AD) dynasties and in Venetian glass from the early fifteenth century. Leonardo Da Vinci was one of the first artists to use cobalt as a brilliant blue pigment in oil paints. The pigment was probably produced by fusing an ore containing cobalt oxide with potash and silica to produce a glass-like material (a smalt), which was then reduced to the powdered pigment. In the sixteenth century, a blue pigment called zaffre was produced from silver–cobalt–bismuth–nickel–arsenate ores in Saxony (IARC, 1991; Donaldson, 2003).

It was not until the twentieth century, however, that cobalt was used for industrial purposes. In 1907, a scientist in the USA, E. Haynes, patented Stellite-type alloys that were very resistant to corrosion and wear at high temperatures (Kirk, 1985). Cobalt was first added to tungsten carbide in 1923 to produce hard metals (Anon., 1989) and permanent magnetic alloys known as Alnicos (cobalt added to alloys of aluminum, nickel and iron) were first described in 1933 (Johnston, 1988; IARC, 1991).

Cobalt is an important metal with many diverse industrial and military applications. Its largest use is in superalloys, which are used primarily to make parts for aircraft gas turbine engines. Cobalt is also an important component of steel when high strength is required, as it increases the tempering resistance of steel; high-strength steels (maraging steels) are used in the aerospace, machine tool and marine equipment industry. Cobalt is also used to make magnets, corrosion- and wear-resistant alloys, high-speed steels, hard-metal and cobalt–diamond tools, cobalt discs and other cutting and grinding tools, catalysts for the petroleum and chemical industries, drying agents for paints, varnishes and inks, ground coats for porcelain enamels, pigments, battery electrodes, steel-belted radial tyres, airbags in automobiles and magnetic recording media (IARC, 1991; Shedd, 2001; Donaldson, 2003).

The major uses of cobalt worldwide in 2003 included: superalloys, 20%; other alloys, 10%; hard metals, 13%; wear-resistant materials, 6%; magnets, 7%; recording materials, 5%; ceramics/enamels/pigments, 12%; batteries, 8%; tyres, paint driers, soaps, 9%; and catalysts, 10% (Hawkins, 2004). According to data from 2002, in the USA, approximately 51% of cobalt was used in superalloys; 8% in cemented carbides (hard metals); 19% in various other metallic uses; and the remaining 22% in a variety of chemical applications (Shedd, 2003).

Cobalt-metal powder (100 mesh or chemical grade) is a common raw material for metal carboxylate production and catalyst manufacture. Fine cobalt powders (400 mesh) are used in hard metals, diamond tools, batteries, magnets, cobalt-containing powdered metal alloys and specialty chemicals. High-purity (99.8%) cobalt briquettes [small lumps or blocks of compressed granular material] are used as raw materials for the production of inorganic cobalt salts and cobalt alloys. Battery-grade cobalt briquettes are used to prepare mixed nitrate solutions for the production of sintered-type nickel hydroxide electrodes. These electrodes are used in nickel–cadmium and nickel–metal hydride batteries. Battery-grade cobalt powders, oxidized as is or after being dissolved in an acid solution,

are used as raw materials to produce cobalt oxide precursors for lithium ion and polymer batteries (OM Group, 2003).

The four most important carbides for the production of hard metals are tungsten carbide (WC), titanium carbide (TiC), tantalum carbide (TaC) and niobium carbide (NbC). Traditionally, cemented carbide (hard-metal) inserts and tools for metal-cutting and metal-working have accounted for the largest percentage of carbide industry sales. However, hard-metal tool consumption in non-metal-working fields, notably in the construction and transportation industries, has grown rapidly. In contrast, the demand for primary materials has been somewhat reduced by the use of recycled hard-metal scrap (Santhanam, 1992; Stoll & Santhanam, 1992).

Cobalt sulfate is the usual source of water-soluble cobalt since it is the most economical salt and shows less tendency to deliquesce or dehydrate than the chloride or nitrate salts. It is used in storage batteries, in cobalt electroplating baths, as a drier for lithographic inks and varnishes, in ceramics, enamels and glazes to prevent discolouring and in cobalt pigments for decorating porcelain (O'Neil, 2001).

Uses of other cobalt compounds are described in detail by IARC (1991).

1.3 Occurrence and exposure

1.3.1 *Natural occurrence*

Cobalt occurs in nature in a widespread but dispersed form in many rocks and soils. The cobalt concentration in the earth's crust is about 20 mg/kg. The largest concentrations of cobalt are found in mafic (igneous rocks rich in magnesium and iron and comparatively low in silica) and ultramafic rocks; the average cobalt content in ultramafic rocks is 270 mg/kg, with a nickel:cobalt ratio of 7. Sedimentary rocks contain varying amounts of cobalt, averaging 4 mg/kg in sandstone, 6 mg/kg in carbonate rocks and 40 mg/kg in clays and shales. Concentrations of cobalt in metamorphic rock depend on the amount of the element in the original igneous or sedimentary source. Cobalt has also been found in meteorites (Donaldson *et al.*, 1986; O'Neil, 2001; Donaldson, 2003).

Cobalt salts occur in nature as a small percentage of other metal deposits, particularly copper; cobalt sulfides, oxides and arsenides are the largest mineral sources of cobalt (Schrauzer, 1989; IARC, 1991; Donaldson, 2003).

1.3.2 *Occupational exposure*

Occupational exposure to aerosols containing cobalt metal or solubilized cobalt compounds may occur during the refining of cobalt, the production of alloys, at various stages in the manufacture of hard metals, the maintenance and resharpening of hard-metal tools and blades and during the manufacture and use of diamond tools containing cobalt (see below). However, only about 15% of cobalt produced is used in cemented carbides (hard metals) and diamond tooling and there are many other potential sources of occupational exposure to cobalt (see Section 4, Table 15).

Several studies have reported occupational exposure to cobalt by measuring concentrations in ambient air in industrial sites where hard-metal and diamond grinding wheels were produced. In addition, analytical methods have been recently standardized for the determination of cobalt concentrations in urine and blood (Kristiansen *et al.*, 1997; White, 1999). It should be noted that many workers inhaling different chemical species of cobalt may also be exposed to nickel, tungsten, chromium, arsenic, molybdenum, beryllium, silica and silicates, asbestos, nitrosamines, diamond powders and iron. Exposure to other substances co-occurring with cobalt have also been reported.

(*a*) *Hard-metal production and use*

Exposure to hard-metal dust takes place at all stages of the production of hard metals, but the highest levels of exposure to cobalt have been reported to occur during the weighing, grinding and finishing phases (Reber & Burckhardt, 1970; McDermott, 1971; National Institute for Occupational Safety and Health, 1981; Sprince *et al.*, 1984; Hartung, 1986; Kusaka *et al.*, 1986; Balmes, 1987; Meyer-Bisch *et al.*, 1989; Auchincloss *et al.*, 1992; Stebbins *et al.*, 1992). For example, in two factories in the USA producing hard metals, peak cobalt concentrations in air taken during weighing, mixing and milling exceeded 500 $\mu g/m^3$ in more than half of all samples (Sprince *et al.*, 1984), and in powder rooms with poorly-regulated control of cobalt dusts, concentrations of cobalt in air ranged between 10 $\mu g/m^3$ and 160 $\mu g/m^3$ (Auchincloss *et al.*, 1992).

Table 5 shows the cobalt concentrations in air determined for all stages in the manufacturing process in a study of exposure to hard metals among hard-metal workers in Japan (Kusaka *et al.*, 1986; Kumagai *et al.*, 1996). The concentrations of cobalt and nickel in air were shown to be distributed lognormally (Kusaka *et al.*, 1992; Kumagai *et al.*, 1997). The workers were further studied with respect to prevalence of asthma in association with exposure to cobalt (Kusaka *et al.*, 1996a,b).

Table 6 summarizes data on cobalt concentrations in workplace air and urine of workers in hard-metal production up to 1986 (presented in the previous monograph on cobalt; IARC, 1991), together with more recent studies.

In a factory producing hard metal in Italy, the mean concentration of cobalt in workplace air on Thursday afternoons was 31.7 ± 33.4 $\mu g/m^3$, thus exceeding the current ACGIH threshold limit value (TLV) for occupational exposure of 20 $\mu g/m^3$ (Scansetti *et al.*, 1998; ACGIH Worldwide®, 2003a). Among hard-metal workers in several small factories in northern Italy, cobalt concentrations in the urine of six operators on machines without aspirators were up to 13 times higher than those in the reference population (Cereda *et al.*, 1994).

A British study reported median concentrations of cobalt in urine of 19 nmol/mmol creatinine in workers in the hard-metal industry and 93 nmol/mmol creatinine in workers manufacturing and handling cobalt powders, salts and pigments in the chemical industry (White & Dyne, 1994).

Table 5. Cobalt concentrations in air in different workshops in the hard-metal industry

Workshop	No. of workers	No. of samples of work-place air	Cobalt concentration (μg/m³)					
			AM[a]	GM[b]	Min.	Max.	GSD_W[c]	GSD_B[d]
Powder preparation								
Rotation	15	60	459	211	7	6390	NA	NA
Full-time	2	12	147	107	26	378	1.88	2.27[e]
Press								
Rubber	8	26	339	233	48	2910	2.77	1.00
Steel	23	34	47	31	6	248	2.43	NA
Shaping	67	179	97	57	4	1160	2.56	1.79
Sintering	37	82	24	13	1	145	1.99	1.99
Blasting	3	7	2	2	1	4	1.88	1.00[e]
Electron discharging	10	18	3	2	1	12	2.69	1.00
Wet grinding	191	517	45	21	1	482	2.30	2.31
Dry grinding without ventilation	1	2	1292	NA	1113	1471	NA	NA

From Kusaka *et al.* (1986); Kumagai *et al.* (1996)
NA, not applicable or not available
[a] AM, arithmetic mean
[b] GM, geometric mean
[c] GSD_W, geometric standard deviation within-worker variation
[d] GSD_B, geometric standard deviation between-worker variation
[e] Because number of workers in this job group was small, the GSD_B value is not reliable.

Concentrations of different tungsten species (W, WC, WO, WO_4^{2-}), cobalt and nickel were studied in air and in urine samples from workers in different areas in a hard-metal factory in Germany. The results are summarized in Tables 7–9 (Kraus *et al.*, 2001).

In addition, the process of depositing carbide coatings, by flame or plasma guns, on to softer substrates to harden their surfaces, may also expose workers to hard metals (Rochat *et al.*, 1987; Figueroa *et al.*, 1992).

Hard metals have applications in tools for machining metals, drawing wires, rods and tubes, rolling or pressing, cutting various materials, drilling rocks, cement, brick, road surfaces and glass, and many other uses in which resistance to wear and corrosion are needed, such as high-speed dental drills, ballpoint pens and tyre studs. During the use of hard-metal tools (e.g. in drilling, cutting, sawing), the levels of exposure to cobalt or hard-metal dust are much lower than those found during their manufacture. However, the grinding of stone and wood with hard-metal tools and the maintenance and sharpening of these tools may release cobalt into the air at concentrations of several hundred micrograms per cubic metre (Mosconi *et al.*, 1994; Sala *et al.*, 1994; Sesana *et al.*, 1994).

Table 6. Biomonitoring of occupational exposure to cobalt in the hard-metal industry

Industry/activity	No. of samples	Sex	Concentration of cobalt in ambient air (mg/m^3)[a]	Concentration of cobalt in blood and urine	Comments	Reference
Hard-metal production (two subgroups)	10	M	a. Mean, 0.09 b. Mean, 0.01 (personal samples)	Blood: a. Mean, 10.5 µg/L b. Mean, 0.7 µg/L Urine: a. Mean, [106] µg/L b. Mean, [~3] µg/L Sampling on Friday pm	Significant correlations: air:urine, $r = 0.79$; air:blood, $r = 0.87$; blood:urine, $r = 0.82$	Alexandersson & Lidums (1979); Alexandersson (1988)
Hard-metal production	7	–	Range, 0.180–0.193	Urine: sampling on Sunday (24 h), mean: 11.7 µg/L	Time of sampling: Monday am for basic exposure level; Friday evening for cumulative exposure level	Pellet *et al.* (1984)
Hard-metal grinding (seven subgroups)	153	–	Up to 61 $\mu g/m^3$ (stationary samples)	Median values for all subgroups: serum, 2.1 µg/L; urine, 18 µg/L	Significant correlation: serum (x)/urine (y) $y = 2.69x + 14.68$	Hartung & Schaller (1985)
Hard-metal tool production (11 subgroups)	170 5	M F	Mean, 28–367 $\mu g/m^3$ (personal samples)	Mean: blood, 3.3–18.7 µg/L; urine, 10–235 µg/L Sampling on Wednesday or Thursday at end of shift	Significant correlations (based on mean values): air (x)/urine (y): $y = 0.67x + 0.9$; air (x)/blood (y): $y = 0.004x + 0.23$; urine (x)/blood (y): $y = 0.0065x + 0.23$	Ichikawa *et al.* (1985)

Table 6 (contd)

Industry/activity	No. of samples	Sex	Concentration of cobalt in ambient air (mg/m^3)[a]	Concentration of cobalt in blood and urine	Comments	Reference
Hard-metal production (six subgroups)	27	–	Breathable dust: range, 0.3–15 with 4–17% cobalt	Mean: serum, 2.0–18.3 µg/L; urine, 6.4–64.3 µg/g creatinine	Significant correlation: serum:urine, $r = 0.93$	Posma & Dijstelberger (1985)
Hard-metal production	26	M	Range, approx. 0.002–0.1; median, approx. 0.01 (personal samples)	Urine: (a) Monday at end of shift, up to 36 µg/L; (b) Friday at end of shift, up to 63 µg/L	Significant correlations: air (x)/urine (y): (a) $y = 0.29x + 0.83$; (b) $y = 0.70x + 0.80$	Scansetti *et al.* (1985)
Machines with aspirators	6–8	–	Mean ± SD: SS: 3.47 ± 2.15 PS: 4.43 ± 2.70	Urine: GM ± GSD[b], 2.66 ± 1.69 µg/L	SS: stationary sample PS: personal sample	Cereda *et al.* (1994)
Machines without aspirators	6–16	–	Mean ± SD: SS: 6.68 ± 2.27 PS: 47.75 ± 3.53	Urine: GM ± GSD[b], 28.50 ± 3.97 µg/L	SS: stationary sample PS: personal sample	Cereda *et al.* (1994)
Hard-metal workers	6	M + F	Mean ± SD (range): Mon: 21.16 ± 17.18 (11–56) Thu: 31.66 ± 33.37 (7–92)	Urine: mean ± SD (range), 13.23 ± 9.92 (2.58–29.8) 30.87 ± 21.94 (8.17–62.6)	Mon: Monday morning Thu: Thursday afternoon	Scansetti *et al.* (1998)

Updated from Angerer & Heinrich (1988); IARC (1991)
–, not stated
[a] Unless stated otherwise
[b] GM, geometric mean; GSD, geometric standard deviation

Table 7. Concentration of cobalt, nickel and tungsten in air in different workshops in the hard-metal industry

Workshop	Sampling method[a]	No. of samples	Concentration in air (μg/m^3)		
			Cobalt	Tungsten	Nickel
Forming	P	5	0.61–2.82	7.8–97.4	0.23–0.76
	S	1	1.32	6.2	0.30
Pressing	P	3	0.87–116.0	5.3–211.0	0.32–3.0
Powder processing	P	4	7.9–64.3	177.0–254.0	0.76–1.65
Production of tungsten carbide	P	1	0.39	19.1	0.40
Sintering	P	1	343.0	12.1	29.6
	S	1	1.3	5.9	0.07
Grinding (wet)	P	1	0.20	3.3	0.13
Grinding (dry)	P	1	0.48	81.3	0.31
Heavy alloy production	P	2	0.85–1.84	125.0–417.0	0.48–2.17
	S	3	0.63–8.50	50.0–163.0	0.72–1.70

From Kraus *et al.* (2001)

[a] P, personal sampling; S, stationary sampling

Coolants are used in the hard-metal industry during the process of grinding of hard-metal tools after sintering and in their maintenance and resharpening. During such operations, the continuous recycling of coolants has been shown to result in increased concentrations of dissolved cobalt in the metal-working liquid and, hence, a greater potential for exposure to (ionic) cobalt in aerosols released from these fluids (Einarsson *et al.*, 1979; Sjögren *et al.*, 1980; Hahtola *et al.*, 2000; Tan *et al.*, 2000). It has been shown that approximately 60% of cobalt trapped in the coolant was in the dissolved form, the remainder being in the form of suspended carbide particles (Stebbins *et al.*, 1992; Linnainmaa *et al.*, 1996). Mists of the coolants in the wet process of grinding hard-metal tools were found to disturb local ventilation systems (Lichtenstein *et al.*, 1975) and, as a result, cobalt concentrations in the air were higher than those from the dry grinding process (Imbrogno & Alborghetti, 1994). Used coolants may contain nitrosamines (Hartung & Spiegelhalder, 1982).

(*b*) *Cobalt-containing diamond tooling*

Diamond tools are used increasingly to cut stone, marble, glass, wood and other materials and to grind or polish various materials, including diamonds. Although these tools are not composed of hard metal, as they do not contain tungsten carbide, they are often considered in the same category. They are also produced by powder metallurgy, whereby microdiamonds are impregnated in a matrix of compacted, extrafine cobalt powder. Consequently, the proportion of cobalt in bonded diamond tools is higher (up to 90%) than in hard metal.

Table 8. Concentration of cobalt, nickel and tungsten in urine of workers in different workshops in the hard-metal industry

Workshop	No. of workers	Metal[a]	Concentration in urine		
			Mean (95% CI) µg/g creatinine	Median µg/g creatinine	Range µg/g creatinine
Forming	23	Co	13.5 (3.7–23.3)	4.2	0.75–106.4
		W	10.7 (6.7–14.6)	9.5	0.33–33.1
		Ni	0.40 (0.19–0.62)	0.3	< DL[b]–2.2
Pressing	30	Co	5.5 (2.9–8.1)	2.8	0.36–35.9
		W	8.6 (4.1–13.1)	6.5	1.5–71.0
		Ni	0.42 (0.28–0.56)	0.4	< DL–1.6
Heavy alloy production	3	Co	1.6 (0.15–3.0)	1.4	1.1–2.2
		W	24.9 (–34.9–84.8)	21.6	2.6–50.5
		Ni	2.9 (–4.8–10.6)	2.2	0.21–6.3
Powder processing	14	Co	28.5 (–5.6–62.7)	11.2	0.75–227.8
		W	12.2 (8.0–16.5)	11.6	2.6–25.1
		Ni	0.53 (0.04–1.0)	0.1	< DL–3.1
Production of tungsten carbide	4	Co	2.1 (–1.9–6.0)	1.1	0.31–5.7
		W	42.1 (4.3–79.9)	48.9	10.0–60.6
		Ni	0.91 (0.13–1.7)	0.8	0.51–1.5
Sintering	6	Co	4.1 (0.12–6.0)	2.6	0.31–9.6
		W	12.5 (–5.7–30.7)	5.5	2.1–46.8
		Ni	0.47 (0.11–0.84)	0.4	< DL–1.0
Grinding	5	Co	2.2 (–0.57–5.0)	1.4	0.19–6.0
		W	94.4 (11.2–177.5)	70.9	10.6–168.6
		Ni	0.25 (0.02–0.48)	0.2	< DL–0.5
Maintenance	2	Co	3.0 (–18.9–24.9)	3.0	1.3–4.7
		W	3.4 (–21.1–27.8)	3.4	1.5–5.3
		Ni	0.63 (–3.5–4.7)	0.6	0.31–1.0

From Kraus *et al.* (2001)
[a] Co, cobalt; W, tungsten; Ni, nickel
[b] DL, detection limit

Exposures to cobalt have been described during the manufacture and use of cobalt–diamond tools. Diamond polishers have been reported to inhale metallic cobalt, iron and silica from so-called cobalt discs during the polishing of diamond jewels (Demedts *et al.*, 1984; Gheysens *et al.*, 1985; Van Cutsem *et al.*, 1987; Van den Eeckhout *et al.*, 1988; Nemery *et al.*, 1990; Van den Oever *et al.*, 1990; Nemery *et al.*, 1992).

Table 9. Monitoring of workplace air and workers' urine for different tungsten species in the hard-metal industry

Workshop	No. of samples[a]	Tungsten species[b]	Air concentration (μg/m³) mean (range)	Urine concentration (μg/g creatinine) mean (range)
Powder processing	4	W	203.5 (177.0–254.0)	13.8 (2.6–21.1)
Forming, pressing, sintering	8	WC	53.5 (5.3–211.0)	9.5 (2.2–33.1)
Production of tungsten carbide	1	WC, WO, W	19.1	59.6
Grinding (wet)	1	WO_4^{3-}	3.3	70.9
Grinding (dry)	1	WO, WC	81.3	10.6

From Kraus *et al.* (2001)

[a] Same number of samples for air and for urine

[b] W, tungsten metal; WC, tungsten carbide; WO, tungsten oxide; WO_4^{3-}, tungstenate

Concentrations of cobalt in the workplace air in one study were below 50 μg/m³ (range, 0.1–45 μg/m³) (Van den Oever *et al.*, 1990). In an Italian factory using diamond wheels to cut wood and stone, mean cobalt concentrations in air were found to be 690 μg/m³ and dropped to 115 μg/m³ after proper ventilation systems were installed (Ferdenzi *et al.*, 1994). Elevated concentrations of cobalt were also reported in the urine of these workers (Van den Oever *et al.*, 1990; Suardi *et al.*, 1994).

(*c*) *Alloys containing cobalt*

Production and use of cobalt alloys gives rise to occupational exposure to cobalt during the welding, grinding and sharpening processes; the welding process with Stellite alloy (cobalt–chromium) was found to generate average concentrations of cobalt in air of 160 μg/m³ (Ferri *et al.*, 1994). A factory producing Stellite tools was reported to have concentrations of cobalt in the air of several hundred micrograms per cubic metre (Simcox *et al.*, 2000), whereas concentrations averaging 9 μg/m³ were noted in another Stellite-producing factory (Kennedy *et al.*, 1995).

(*d*) *Cobalt pigments*

Porcelain plate painters in Denmark have been exposed for many decades to cobalt (insoluble cobalt–aluminate spinel or soluble cobalt–zinc silicate) at concentrations which exceeded the hygiene standard by 1.3–172-fold (Tüchsen *et al.*, 1996). During the period 1982–92, the Danish surveillance programme showed a reduction in exposure to cobalt both in terms of concentrations in air and urine; the concentration of cobalt in air decreased from 1356 nmol/m³ [80 μg/m³] to 454 nmol/m³ [26 μg/m³], and that in urine of workers from 100-fold to 10-fold above the median concentration of unexposed control subjects (Christensen & Poulsen, 1994; Christensen, 1995; Poulsen *et al.*, 1995).

A group of workers producing cloisonné [enamel ware] in Japan and exposed to lead, chromium, cadmium, manganese, antimony, copper and cobalt compounds showed peak cobalt concentrations in blood that were twofold higher compared with the referent group, although cobalt concentrations in urine were similar (Arai *et al.*, 1994).

(*e*) *Production of cobalt metal and cobalt salts*

In a factory in Belgium engaged in hydrometallurgical purification, workers were exposed to cobalt metal, cobalt oxide and cobalt salts without being exposed to tungsten, titanium, iron or silica, or their carbides, or to diamond. The mean concentration of cobalt in the workplace air was 127.5 μg/m^3 (median, 84.5 μg/m^3; range, 2–7700 μg/m^3). Cobalt concentrations in urine samples from workers taken after the workshift on Fridays averaged 69.8 μg/g creatinine (median, 72.4 μg/g; range, 1.6–2038 μg/g creatinine) (Swennen *et al.*, 1993). Cobalt concentrations in urine at the end of the workshift correlated well with workers' exposure on an individual basis to cobalt metal and cobalt salts, but not with exposure to cobalt oxide. Cobalt concentrations of 20 and 50 μg/m^3 in air would be expected to lead to cobalt concentrations in urine of 18.2 and 32.4 μg/g creatinine, respectively (Lison *et al.*, 1994).

Recycling of batteries for the purpose of recovering cobalt, nickel, chromium and cadmium was found to result in cobalt concentrations in workplace air of up to 10 μg/m^3 (Hengstler *et al.*, 2003).

Workers in a factory in the Russian Federation producing cobalt acetate, chloride, nitrate and sulfates were reported to be exposed to cobalt in dust at concentrations of 0.05–50 mg/m^3 (Talakin *et al.*, 1991). In a nickel refinery also in the Russian Federation, exposures to airborne cobalt of up to 4 mg/m^3 were reported; nickel and cobalt concentrations were strongly correlated, although inhaled concentrations of nickel were far greater than those of cobalt (Thomassen *et al.*, 1999).

In a cobalt plant in Kokkola, Finland, workers were potentially exposed to metallic cobalt and cobalt sulfates, carbonates, oxides and hydroxides (Linna *et al.*, 2003). The highest concentration of cobalt in urine was recorded in a worker in the reduction department (16 000 nmol/L [943 μg/L]). Among workers in the solution, purification and chemical departments, cobalt concentrations in urine ranging from 300 to 2000 nmol/L [18 to 118 μg/L] were reported, while mean concentrations of cobalt in the air of all work areas were below 100 μg/m^3.

In a plant in South Africa converting cobalt metal to cobalt oxide, the highest concentrations of cobalt in ambient air and in urine samples of workers were 9.9 mg/m^3 and 712 μg/g creatinine, respectively (Coombs, 1996).

High concentrations of cobalt, as well as antimony, arsenic, cadmium, chromium, lanthanum, lead and selenium, were reported in the lungs of a group of smelter workers in Sweden (Gerhardsson & Nordberg, 1993). Workers from a smelter, a petroleum refinery and a chemical plant in the USA were found to have significantly lower concentrations of cobalt in the seminal plasma, while concentrations of zinc, copper and nickel were high compared with a referent group of hospital workers (Dawson *et al.*, 2000).

(*f*) *Other exposures*

In the United Kingdom, workers in metal thermal spraying were found to inhale cobalt, chromium and nickel. Monitoring of the workplace air and the urine of workers showed concentrations of cobalt in air of 20–30 μg/m^3 and in urine of 10–20 μmol/mol creatinine, a range 10- to 20-fold higher than in unexposed controls (Chadwick *et al.*, 1997).

Non-occupational exposure to cobalt arises from surgical implants and dental prostheses, and from contact with metallic objects, e.g. jewellery. A slight increase in mean cobalt concentrations was reported in the urine of patients with cobalt-alloy knee and hip prostheses (Sunderman *et al.*, 1989).

1.3.3 *Environmental exposure*

(*a*) *Air*

Cobalt is released into the air from volcanoes and burning fuels (coal, oil). Bertine and Goldberg (1971) estimated a concentration of cobalt of 5 mg/kg in coal and 0.2 mg/kg in oil. The active volcano Mt. Erebus in Antarctica releases considerable amounts of trace elements into the environment, including cobalt (Kyle & Meeker, 1990; Hamilton, 1994). In Mumbai, India, Sadasivan and Negi (1990) found mean concentrations of cobalt in atmospheric aerosols of 1.1 ± 1.5 ng/m^3 (range, 0.3–2.3 ng/m^3), originating from iron debris in the soil. Between 1962 and 1974, average cobalt concentrations in the air in the United Kingdom declined significantly in all but one of seven sampling sites (Hamilton, 1994). Atmospheric concentrations of cobalt in rural areas of developed countries are usually below 1 ng/m^3 (Hamilton, 1994).

(*b*) *Water and sediments*

Cobalt concentrations in sea water range from 0.01–4 μg/L and in fresh and ground waters from 0.1–10 μg/L (Nilsson *et al.*, 1985). Of 720 river water samples examined in the USA, 37% contained traces of cobalt, in the range of 1–5 μg/L, 5 μg/L being the limit of solubility. Because cobalt is present only in low concentrations, no maximal level has been set for drinking-water (Calabrese *et al.*, 1985).

Cobalt concentrations in sediments may vary from < 6 ppm (low) to > 125 ppm (very high) (Hamilton, 1994).

(*c*) *Soils and plants*

Cobalt is omnipresent in soil, but is far from being distributed evenly. Apparently there exists a correlation between the content of cobalt in soil and in the parent rock; as a consequence, soils that are geochemically rich or poor in cobalt can be recognized. Cobalt concentrations in most soils range from 0.1–50 ppm and the amount of cobalt taken up by plants from 0.1 to 2 ppm (Nilsson *et al.*, 1985; Hamilton, 1994). However, industrial pollution may lead to much higher concentrations; close to a hard-metal (tool grinding)

factory in the USA, soil was contaminated with cobalt at concentrations up to 12 700 mg/kg (Abraham & Hunt, 1995).

Lack of cobalt in soils results in vitamin B_{12} deficiency in ruminants (Domingo, 1989; Hamilton, 1994).

(*d*) *Foods and beverages*

Individual intake of cobalt from food is somewhat variable, but typically in the range 10–100 μg/day. Higher intake may result from taking some vitamin preparations (IARC, 1991).

1.4 Regulations and guidelines

Regulations and guidelines for occupational exposure to cobalt in some countries are presented in Table 10. ACGIH Worldwide® (2003b) recommends a semi-quantitative biological exposure index (BEI) of 15 μg/L in urine and 1 μg/L in blood, and recommends monitoring cobalt in urine or blood of individuals at the end of their last shift of the working week as an indicator of recent exposure.

2. Studies of Cancer in Humans

2.1 Hard-metal industry

Four mortality studies have been carried out in two cohorts of workers from the hard metal industry in Sweden and France. The key findings are summarized in Table 11.

Hogstedt and Alexandersson (1990) reported on 3163 male workers, each with at least 1 year of occupational exposure to hard-metal dust at one of three hard-metal manufacturing plants in Sweden in 1940–82 and who were followed during the period 1951–82. There were four categories of exposure (with estimated concentrations of cobalt in ambient air prior to 1970 given in parentheses for each category): occasionally present in rooms where hard metal was handled (< 2 μg/m^3 cobalt); continuously present in rooms where hard metal was handled, but personal work not involving hard metal (1–5 μg/m^3 cobalt); manufacturing hard-metal objects (10–30 μg/m^3 cobalt); and exposed to cobalt in powder form when manufacturing hard-metal objects (60–11 000 μg/m^3 cobalt). The workers were also exposed to a number of other substances used in the production of hard metal, such as tungsten carbide. There were 292 deaths among persons under 80 years of age during the study period (standardized mortality ratio [SMR], 0.96; 95% confidence interval [CI], 0.85–1.07) and 73 cancer deaths (SMR, 1.05; 95% CI, 0.82–1.32). Seventeen deaths from lung cancer were observed (SMR, 1.34; 95% CI, 0.77–2.13). Comparing the high versus low categories of exposure intensity, SMRs were similar. With regard to latency (time since first exposure), the excess was higher in the subcohort with more than 20 years since first exposure. Among workers with more than 10 years of employment and more than 20 years

Table 10. Occupational exposure limit values and guidelines for cobalt

Country or region	Concentration (mg/m^3)[a]	Interpretation[b]	Carcinogen category[c]
Australia	0.05	TWA	Sen
Belgium	0.02	TWA	
Canada			
Alberta	0.05	TWA	
	0.1	STEL	
Ontario	0.02	TWA	
Quebec	0.02	TWA	A3
China	0.05	TWA	
	0.1	STEL	
Finland	0.05	TWA	
Germany	0.5[d]	TWA (TRK)	2; Sah
Ireland	0.1	TWA	
Japan	0.05	TWA	2B; Aw1S1
	0.2	STEL	
Malaysia	0.02	TWA	
Mexico	0.1	TWA	A3
Netherlands	0.02	TWA	
New Zealand	0.05	TWA	A3
Norway	0.02	TWA	Sen
Poland	0.05	TWA	
	0.2	STEL	
South Africa	0.1	TWA	
Spain	0.02	TWA	
Sweden	0.05	TWA	Sen
Switzerland	0.1	TWA	Sen; K
United Kingdom	0.1	TWA (MEL)	
USA[e]			
ACGIH	0.02	TWA (TLV)	A3
NIOSH	0.05	TWA (REL)	
OSHA	0.1	TWA (PEL)	

From Deutsche Forschungsgemeinschaft (2002); Health and Safety Executive (2002); ACGIH Worldwide® (2003a,b,c); Suva (2003)

[a] Most countries specify that the exposure limit applies to cobalt 'as Co'.

[b] TWA, 8-h time-weighted average; STEL, 10–15-min short-term exposure limit; TRK, technical correct concentration; MEL, maximum exposure level; TLV, threshold limit value; REL, recommended exposure level; PEL, permissible exposure level

[c] Sen, sensitizer; A3, confirmed animal carcinogen with unknown relevance to humans; 2, considered to be carcinogenic to humans; Sah, danger of sensitization of the airways and the skin; 2B, possibly carcinogenic to humans: substance with less evidence; Aw1S1, airway sensitizer; K, carcinogenic

[d] Cobalt metal used in the production of cobalt powder and catalysts, hard metal (tungsten carbide) and magnet production (processing of powder, machine pressing and mechanical processing of unsintered articles); all other uses have a TRK of 0.1 mg/m^3.

[e] ACGIH, American Conference of Governmental Industrial Hygienists; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Health and Safety Administration

Table 11. Cohort studies of lung cancer in workers in the hard-metal and cobalt industry

Reference, plants	Cohort characteristics	No. of deaths	Exposure categories	Observed/ expected or cases/ controls	Relative risk (95% CI)	Comments
Hard-metal industry						
Hogstedt & Alexandersson (1990) 3 factories in Sweden	3163 male workers; follow-up, 1951–82	17 deaths		Obs/Exp	**SMR**	No information on smoking
			Whole cohort	17	1.34 [0.77–2.13]	
			Low exposure	11/8.4	1.31 [0.65–2.34]	
			High exposure	6/4.3	1.39 [0.51–3.04]	
			≥ 10 years of exposure and > 20 years since first exposure	7/2.5	2.78 [1.11–5.72]	
			High exposure			
			< 20 years latency	2/2.6	0.77 [0.09–2.78]	
			≥ 20 years latency	4/1.7	2.35 [0.64–6.02]	
Lasfargues *et al.* (1994) 1 factory in France	709 male workers employed > 1 year; follow-up, 1956–89; vital status, 89.4%; cause of death, 90.7%	10 deaths	Whole cohort	10/4.69	2.13 [1.02–3.93]	National reference. Proportion of smokers comparable with a sample of the French male population
			Duration of employment (years)			
			1–9	7/2.07	3.39 [1.36–6.98]	
			10–19	1/0.81	1.23 [0.03–6.84]	
			≥ 20	1/0.40	2.52 [0.06–14.02]	
			Time since first employment (years)			
			1–9	1/0.54	1.86 [0.05–10.39]	
			10–19	5/1.37	3.65 [1.19–8.53]	
			≥ 20	3/1.38	2.17 [0.45–6.34]	
			Degree of exposure			
			Non-exposed	1/0.66	1.52 [0.04–8.48]	
			Low	0/0.71	0.00 [0.00–5.18]	
			Medium	3/2.08	1.44 [0.30–4.21]	
			High	6/1.19	5.03 [1.85–10.95]	

Table 11 (contd)

Reference, plants	Cohort characteristics	No. of deaths	Exposure categories	Observed/ expected or cases/ controls	Relative risk (95% CI)	Comments
Moulin *et al.* (1998) 10 factories in France	7459 workers (5777 men, 1682 women); follow-up, 1968–91; vital status, 90.8%; cause of death, 96.8%	63 deaths	Whole cohort	Obs/Exp 63/48.59	1.30 [1.00–1.66]	Information on smoking for 80% of participants but no adjustment for smoking. Includes the factory studied by Lasfargues *et al.* (1994)
	Nested case–control study; 61 cases (59 men, 2 women) and 180 controls (174 men, 6 women) followed-up at the time the case died and employed > 3 months, matched by gender and age		**Cobalt with tungsten carbide**	Cases/ controls	**Odds ratio**	
			Levels 2–9/levels 0–1	35/81	1.9 (1.03–3.6)	
			Levels			
			0–1	26/99	1.0	
			2–3	8/12	3.4 (1.2–9.6)	
			4–5	19/55	1.5 (0.8–3.1)	
			6–9	8/14	2.8 (0.96–8.1)	
			p for trend		0.08	
			Duration of exposure (levels ≥ 2)			
			Non-exposed	26/99	1.0	
			≤ 10 years	19/52	1.6 (0.8–3.3)	
			10–20 years	12/20	2.8 (1.1–6.8)	
			> 20 years	4/9	2.0 (0.5–8.5)	
			p for trend		0.03	
			Unweighted cumulative dose[a]			
			< 32	6/46	1.0	
			32–142	16/43	2.6 (0.9–7.5)	
			143–299	16/45	2.6 (1.5–11.5)	
			> 299	23/46	4.1 (1.5–11.5)	
			p for trend		0.01	

Table 11 (contd)

Reference, plants	Cohort characteristics	No. of deaths	Exposure categories	Observed/ expected or cases/ controls	Relative risk (95% CI)	Comments
Moulin *et al.* (1998) (contd)			*Frequency-weighted cumulative dose*[a]	Cases/ controls		
			< 4	8/45	1.0	
			4–27	20/45	2.3 (0.9–6.1)	
			27–164	14/45	1.9 (0.7–5.2)	
			> 164	19/45	2.7 (1.0–7.3)	
			p for trend		0.08	
			Other exposure to cobalt (duration of exposure to levels ≥ 2)	15/30	2.2 (0.99–4.9)	Cobalt alone or simultaneously with agents other than tungsten carbide
Wild *et al.* (2000) 1 factory in France	2860 workers (2216 men, 644 women); follow-up, 1968–92; cause of death, 96%	46 deaths		Obs/Exp	**SMR** Men	Not adjusted for smoking
			Whole cohort	46/27.11	1.70 (1.24–2.26)	
			Hard-metal dust intensity score ≥ 2	26/12.89	2.02 (1.32–2.96)	
			Before sintering	9/3.72	2.42 (1.10–4.59)	
			After sintering	5/3.91	1.28 (0.41–2.98)	
			Per 10 years of exposure to unsintered hard-metal dust		1.43 (1.03–1.98)	Poisson regression adjusted for smoking and asbestos, PAH, silica, nickel and chromium compounds
			Sintered hard metal dust (yes/no)		0.75 (0.37–1.53)	

Table 11 (contd)

Reference, plants	Cohort characteristics	No. of deaths or cases	Exposure categories	Observed/ expected or cases/ controls	Relative risk (95% CI)	Comments
Cobalt production industry						
Moulin *et al.* (1993) 1 electro-chemical plant in France	1148 male workers employed 1950–80; follow-up until 1988; vital status, 99%	8 deaths	Exclusively employed in cobalt production	3/2.58	1.16 (0.24–3.40)	Not adjusted for smoking
			Ever employed in cobalt production	4/3.38	1.18 (0.32–3.03)	
Other cobalt compounds						
					SIR	
Tüchsen *et al.* (1996) 2 porcelain plants in Denmark	1394 female workers (874 exposed; 520 not exposed) employed in the plate underglazing departments 1943–92	15 cases (8 exposed; 7 not exposed)	Exposed to cobalt	8/3.41	2.35 [1.01–4.62]	No information on smoking
			Not exposed to cobalt	7/3.51	1.99 [0.80–4.11]	

PAH, polycyclic aromatic hydrocarbon
[a] Cumulative doses expressed in months × levels

since first exposure, a significant excess of mortality from lung cancer was found (seven cases observed; SMR, 2.78; 95% CI, 1.11–5.72). In addition, there were four deaths from pulmonary fibrosis in this cohort (1.4% of all deaths, which the authors noted to be higher than the national proportion of 0.2%). A survey carried out at the end of the 1970s among hard-metal workers in Sweden showed that their smoking habits were not different from those of the male Swedish population in general (Alexandersson, 1979). [The Working Group noted the small number of exposed lung cancer cases, the lack of adjustment for other carcinogenic exposures and the absence of a positive relationship between intensity of exposure and lung cancer risk.]

A cohort mortality study was carried out among workers at a plant producing hard metals in France (Lasfargues *et al.*, 1994). Seven hundred and nine male workers with at least 1 year of employment were included in the cohort and were followed from 1956 to 1989. Job histories were obtained from company records; however, before 1970 these histories were often missing. Using concentrations of cobalt measured in dust and in urine of workers in 1983, and taking into account improvements in working conditions over time, four categories of exposure were defined: not exposed directly to hard-metal dust; low exposure (cobalt in dust, < 10 μg/m^3; cobalt in urine, 0.01–0.02 μmol/L); medium exposure (cobalt in dust, 15–40 μg/m^3; cobalt in urine, 0.01–0.10 μmol/L); high exposure (atmospheric mean concentrations of cobalt, > 50 μg/m^3; cobalt in urine, 0.02–0.28 μmol/L). Workers who had been employed in jobs with different degrees of exposure were categorized according to their highest exposure and possible previous exposure at other plants was also considered. Of the 709 cohort members, 634 (89.4%) were alive and 295 were still employed at the end of follow-up. Smoking was ascertained for 81% of the workers and 69% of the deceased. The overall mortality did not differ from that expected (75 deaths; SMR, 1.05; 95% CI, 0.82–1.31) whereas mortality due to lung cancer was in excess (10 deaths; SMR, 2.13; 95% CI, 1.02–3.93). This excess was highest among workers employed in the areas with the highest exposures to cobalt (six deaths; SMR, 5.03; 95% CI, 1.85–10.95).

Following the report by Lasfargues *et al.* (1994) described above, an industry-wide mortality study on the association between lung cancer and occupational exposure to cobalt and tungsten carbide was carried out in the hard-metal industry in France (Moulin *et al.*, 1998). The cohort comprised 7459 workers (5777 men, 1682 women) from 10 factories, including the one previously studied by Lasfargues *et al.* (1994), from the time each factory opened (between 1945 and 1965) until 31 December 1991. The minimum time of employment was 3 months in nine factories and 1 year in the factory previously studied (Lasfargues *et al.*, 1994). The mortality follow-up period was 1968–91. A total of 1131 workers were considered to be lost to follow-up; of these, 875 were born outside France. The causes of the 684 registered deaths were ascertained from death certificates (633 subjects) and from medical records (29 subjects), but were unknown for 22 subjects (3.2%). The SMR for all causes of mortality was 0.93 (684 deaths; 95% CI, 0.87–1.01), and mortality for lung cancer was increased (63 deaths; SMR, 1.30; 95% CI, 1.00–1.66) when compared with national death rates. [The loss to follow-up will underestimate the SMRs,

although analyses from the nested case–control study will probably be less affected by this bias.]

Sixty-one cases (i.e. deaths from lung cancer) and 180 controls were included in a nested case–control study (Moulin *et al.*, 1998). Three controls per case were sampled among cohort participants: (a) under follow-up on the date that the case died, having completed 3 months of employment and known to be alive on that date; and (b) of the same gender and with the same date of birth ± 6 months. Job histories were drawn from administrative records and information on job histories was complemented by interviews with colleagues who were not aware of the case or control status of the subjects. Occupational exposure of cases and controls was obtained using a job–exposure matrix involving 320 job periods and semi-quantitative exposure intensity scores from 0 to 9. Exposure was assessed as (i) simultaneous exposure to cobalt and tungsten carbide specific to hard-metal manufacture and (ii) other exposure to cobalt resulting from other production activities. Exposure to cobalt with tungsten carbide was analysed using the maximum intensity score coded at any period of the job history, the duration of exposure at an intensity of ≥ 2 and the estimated cumulative exposure. Cumulative exposure was expressed as either an unweighted (intensity × duration) or a frequency-weighted (intensity × duration × frequency) score. The cumulative exposure scores were divided into quartiles of the exposure distribution among controls after exposure to cobalt had been classified as exposed versus unexposed. Exposure scores for each risk were based on information up to 10 years prior to the death of the case. Information on smoking habits (defined as never, former or current smokers) was obtained by interviewing colleagues, relatives and the subjects themselves. For analysis, each subject was classified as an ever versus never smoker. Information on smoking habits was available for 80% of the study population. The effect of possible confounders, including potential carcinogens listed in the job–exposure matrix (assessed as 'yes' or 'no'), socioeconomic level and smoking, was assessed using a multiple logistic model.

The odds ratio for workers exposed to cobalt and tungsten carbide was 1.93 (95% CI, 1.03–3.62) for exposure levels 2–9 versus levels 0–1. The odds ratio for cobalt with tungsten carbide increased with duration of exposure and unweighted cumulative dose, but less clearly with level of exposure or frequency-weighted cumulative dose. Exposure to cobalt and tungsten before sintering was associated with an elevated risk (odds ratio, 1.69; 95% CI, 0.88–3.27), which increased significantly with frequency-weighted cumulative exposure ($p = 0.03$). The odds ratio for exposure to cobalt and tungsten after sintering was lower (1.26; 95% CI, 0.66–2.40) and no significant trend was observed for cumulative exposure. Adjustment for exposure to known or suspected carcinogens did not change the results. Adjustment for smoking in the 80% subset with complete smoking data resulted in a slightly higher odds ratio (2.6; 95% CI, 1.16–5.82; versus 2.29; 95% CI, 1.08–4.88). The odds ratio for cobalt alone or with exposures other than to tungsten carbide was 2.21 (95% CI, 0.99–4.90) in a model with only indicators of duration of exposure to cobalt with tungsten carbide.

A study in addition to that of Moulin *et al.* (1998) was conducted in the largest plant already included in the multicentre cohort and used the same job–exposure matrix but made

use of the more detailed job histories available (Wild *et al.*, 2000). In this study, which included follow-up from 1968 to 1992, mortality from all causes among 2860 subjects was close to the expected number (399 deaths; SMR for men and women combined, 1.02; 95% CI, 0.92–1.13). Mortality from lung cancer was increased among men (46 deaths; SMR, 1.70; 95% CI, 1.24–2.26). The SMR for exposure to hard-metal dust at an intensity score ≥ 2 was increased (26 deaths; SMR, 2.02; 95% CI, 1.32–2.96). Lung cancer mortality was higher than expected in those working in hard-metal production before sintering (nine deaths; SMR, 2.42; 95% CI, 1.10–4.59); after sintering, the SMR was 1.28 (five deaths; 95% CI, 0.41–2.98). In a Poisson regression model (Table 11) including terms for smoking and other occupational carcinogens, the risk for lung cancer increased with duration of exposure to cobalt with tungsten carbide before sintering (1.43 per 10-year period); there was no evidence of risk from exposure to sintered hard-metal dust.

2.2 Cobalt production industry

Moulin *et al.* (1993) studied the mortality of a cohort of 1148 workers in a cobalt electrochemical plant in France which produced cobalt and sodium by electrochemistry, extending the follow-up of an earlier study (Mur *et al.*, 1987; reported in IARC, 1991). The cohort included all the men who had worked in this plant for a minimum of 1 year between 1950 and 1980. The vital status of the members of the cohort was ascertained up to the end of 1988, and was obtained for 99% of French-born workers using information provided by the registry office of their place of birth. Due to difficulties in tracing workers born outside France, results are presented here only for French-born workers ($n = 870$).

The SMR for all causes of death was 0.95 (247 deaths; 95% CI, 0.83–1.08) and that for all cancer deaths was 1.00 (72 deaths; 95% CI, 0.78–1.26). The SMR for lung cancer mortality was 1.16 (three deaths; 95% CI, 0.24–3.40) among workers employed exclusively in cobalt production and 1.18 (four deaths; 95% CI, 0.32–3.03) for workers ever employed in cobalt production. For workers who worked exclusively as maintenance workers, the SMR for lung cancer was 2.41 (two deaths; 95% CI, 0.97–4.97) and, for those ever employed as maintenance workers, it was 2.58 (eight deaths; 95% CI, 1.12–5.09). There was evidence for an increased risk in this group of workers for those employed more than 10 years in cobalt production and for 30 years or more since first employment in cobalt production. [The Working Group noted that this might be explained by other carcinogenic exposures such as smoking or other occupational exposures such as asbestos.]

2.3 Other cobalt compounds

A study was conducted among 874 women occupationally exposed to poorly soluble cobalt–aluminate spinel and 520 women not exposed to cobalt in two porcelain factories in Denmark (Tüchsen *et al.*, 1996). The period of follow-up was from 1943 (time of first employment) to 1992. Vital status was assessed through the national population register and incident cancer cases were traced through the national cancer register. The observed

deaths and incident cancer cases were compared with the expected numbers based on national rates for all Danish women. Cobalt concentrations in air in this plant were high (often > 1000 μg/m³). During the follow-up period, 127 cancer cases were diagnosed in the cohort. The overall cancer incidence was slightly elevated among the exposed women (67 observed; standardized incidence ratio [SIR], 1.20; 95% CI, 0.93–1.52) and close to unity in the reference group (60 observed; SIR, 0.99 [95% CI, 0.76–1.27]). Compared with the national reference rate, both exposed women (eight observed; SIR, 2.35; 95% CI, 1.01–4.62) and the reference group (seven observed; SIR, 1.99; 95% CI, 0.80–4.11) had an increased risk for lung cancer. However, the exposed group had a relative risk ratio of 1.2 (95% CI, 0.4–3.8) when compared with the reference group.

No relation with duration or intensity of exposure was found. The influence of smoking could not be taken into account in this study. Among the eight cases of lung cancer identified in the exposed cohort, three had been exposed to cobalt spinel for less than 3 months. [This study did not provide evidence of an increased risk of lung cancer associated with exposure to cobalt spinel.]

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

There are no data relative to carcinogenicity by inhalation of cobalt metal, cobalt-metal powder or cobalt alloys.

3.1.1 *Mouse*

In a study undertaken by the National Toxicology Program (1998), groups of 50 male and 50 female B6C3F$_1$ mice, 6 weeks of age, were exposed to aqueous aerosols of 0, 0.3, 1 or 3 mg/m³ cobalt sulfate heptahydrate (purity, ≈ 99%; mass median aerodynamic diameter (MMAD), 1.4–1.6 μm; geometric standard deviation (GSD), 2.1–2.2 μm) for 6 h per day on 5 days per week for 105 weeks. No adverse effects on survival were observed in treated males or females compared with chamber controls (survival rates: 22/50 (control), 31/50 (low dose), 24/50 (mid dose) or 20/50 (high dose) in males and 34/50, 37/50, 32/50 or 28/50 in females, respectively; survival times: 662, 695, 670 or 643 days in males and 694, 713, 685 or 680 days in females, respectively). Mean body weights increased in all treated females from week 20 to 105 and decreased in males exposed to the high dose from week 96 to 105 when compared with chamber controls. The incidence of neoplasms and non-neoplastic lesions of the lung is reported in Table 12. Exposure to cobalt sulfate heptahydrate caused a concentration-related increase in benign and malignant alveolar/bronchiolar neoplasms in male and female mice. All the alveolar/bronchiolar proliferative lesions observed within the lungs of exposed mice were typical of those arising spontaneously. However, exposure to cobalt did not cause an increased incidence of neoplasms in other tissues (National Toxicology Program, 1998; Bucher *et al.*, 1999).

Table 12. Incidence of neoplasms and non-neoplastic lesions of the lung in mice in a 2-year inhalation study of cobalt sulfate heptahydrate

Lesions observed	No. of mice exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Males				
Total no. examined microscopically	50	50	50	50
Infiltration cellular, diffuse, histiocyte	1 (3.0)[a]	2 (3.0)	4 (2.3)	10[b] (1.5)
Infiltration cellular, focal, histiocyte	10 (2.7)	5 (2.6)	8 (3.0)	17 (2.7)
Bronchus, cytoplasmic vacuolization	0	18[b] (1.0)	34[b] (1.0)	38[b] (1.0)
Alveolar epithelium hyperplasia	0	4 (2.3)	4 (1.8)	4 (2.3)
Alveolar/bronchiolar adenoma	9	12	13	18[c]
Alveolar/bronchiolar carcinoma	4	5	7	11[c]
Alveolar/bronchiolar adenoma or carcinoma	11	14	19	28[b]
Females				
Total no. examined microscopically	50	50	50	50
Infiltration cellular, diffuse, histiocyte	0	0	0	4 (3.3)
Infiltration cellular, focal, histiocyte	2 (2.0)	5 (1.8)	7 (2.9)	10[c] (2.4)
Bronchus, cytoplasmic vacuolization	0	6[c] (1.0)	31[b] (1.0)	43[b] (1.0)
Alveolar epithelium hyperplasia	2 (1.5)	3 (1.3)	0	5 (2.0)
Alveolar/bronchiolar adenoma	3	6	9	10[c]
Alveolar/bronchiolar carcinoma	1	1	4	9[b]
Alveolar/bronchiolar adenoma or carcinoma	4	7	13[c]	18[b]

From National Toxicology Program (1998)

[a] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked

[b] Significantly different ($p \leq 0.01$) from the chamber control group by the logistic regression test

[c] Significantly different ($p \leq 0.05$) from the chamber control group by the logistic regression test

3.1.2 *Rat*

In a study undertaken by the National Toxicology Program (1998), groups of 50 male and 50 female Fischer 344/N rats, 6 weeks of age, were exposed to aqueous aerosols of 0, 0.3, 1 or 3 mg/m^3 cobalt sulfate heptahydrate (purity, ≈ 99%; MMAD, 1.4–1.6 μm; GSD, 2.1–2.2 μm) for 6 h per day on 5 days per week for 105 weeks. No adverse effects on mean body weights nor on survival were observed in treated males or females compared with chamber controls (survival rates: 17/50 (control), 15/50 (low dose), 21/50 (mid dose) or 15/50 (high dose) in males and 28/50, 25/49, 26/50 or 30/50 in females, respectively; survival times: 648, 655, 663 or 643 days in males and 699, 677, 691 or 684 days in females, respectively). Exposure to cobalt sulfate heptahydrate caused a concentration-related increase in the incidence of benign and malignant alveolar bronchiolar neoplasms

in male and female rats and benign and malignant pheochromocytomas in female rats. However, exposure to cobalt sulfate did not cause an increased incidence of neoplasms in other tissues. The incidence of neoplasms and non-neoplastic lesions is reported in Table 13. In rats exposed to cobalt sulfate heptahydrate by inhalation, a broad spectrum of inflammatory and proliferative pulmonary lesions was observed. While many of these tumours were highly cellular and morphologically similar to those arising spontaneously, others, in contrast to those seen in mice, were predominantly fibrotic, squamous or mixtures of alveolar/bronchiolar epithelium and squamous or fibrous components. Benign neoplasms typical of those arising spontaneously were generally distinct masses that often compressed surrounding tissue. Malignant alveolar/bronchiolar neoplasms had similar cellular patterns but were generally larger and had one or more of the following histological features; heterogeneous growth pattern, cellular pleomorphism and/or atypia, and local invasion or metastasis. In addition to these more typical proliferative lesions, there were 'fibroproliferative' lesions ranging from less than 1 mm to greater than 1 cm in diameter. Small lesions with modest amounts of peripheral epithelial proliferation were diagnosed as atypical hyperplasia, while larger lesions with florid epithelial proliferation, marked cellular pleomorphism, and/or local invasion were diagnosed as alveolar/bronchiolar carcinomas. While squamous epithelium is not normally observed within the lung, squamous metaplasia of alveolar/bronchiolar epithelium is a relatively common response to pulmonary injury and occurred in a number of rats in this study. Squamous metaplasia consisted of small clusters of alveoli in which the normal epithelium was replaced by multiple layers of flattened squamous epithelial cells that occasionally formed keratin. One male and one female each had a large cystic squamous lesion rimmed by a variably thick band of friable squamous epithelium with a large central core of keratin. These lesions were diagnosed as cysts. In two exposed females, proliferative squamous lesions had cystic areas but also more solid areas of pleomorphic cells and invasion into the adjacent lung; these lesions were considered to be squamous-cell carcinomas. In all groups of male and female rats exposed to cobalt sulfate heptahydrate, the incidence of alveolar proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation and interstitial fibrosis was significantly greater than in the chamber controls. Exposure to cobalt sulfate heptahydrate caused a concentration-related increased incidence of benign and malignant pheochromocytomas in female rats. Although a very common spontaneous neoplasm in male Fischer 344/N rats, pheochromocytomas have a lower spontaneous occurrence in females. The marginally-increased incidence of pheochromocytomas in males was considered an uncertain finding because it occurred only in the group exposed to 1.0 mg/m^3 and was not supported by increased incidence or severity of hyperplasia (National Toxicology Program, 1998; Bucher *et al.*, 1999).

Table 13. Incidence of neoplasms and non-neoplastic lesions of the lung in rats and of the adrenal medulla in female rats in a 2-year inhalation study of cobalt sulfate heptahydrate

Lesions observed	No. of rats exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Males				
Lung				
No. examined microscopically	50	50	48	50
Alveolar epithelium, hyperplasia	9 (1.8)[a]	20[b] (2.0)	20[b] (2.1)	23[c] (2.0)
Alveolar epithelium, hyperplasia, atypical	0	2 (3.0)	2 (3.0)	2 (4.0)
Metaplasia, squamous	0	1 (1.0)	4 (2.0)	2 (3.0)
Alveolar epithelium, metaplasia	0	50[c] (1.9)	48[c] (3.1)	49[c] (3.7)
Inflammation, granulomatous	2 (1.0)	50[c] (1.9)	48[c] (3.1)	50[c] (3.7)
Interstitium, fibrosis	1 (1.0)	50[c] (1.9)	48[c] (3.1)	49[c] (3.7)
Proteinosis	0	16[c] (1.4)	40[c] (2.3)	47[c] (3.4)
Cyst	0	0	0	1 (4.0)
Alveolar/bronchiolar adenoma	1	4	1	6
Alveolar/bronchiolar carcinoma	0	0	3	1
Alveolar/bronchiolar adenoma or carcinoma	1	4	4	7/50[b]
Females				
Lung				
No. examined microscopically	50	49	50	50
Alveolar epithelium, hyperplasia	15 (1.4)	7 (1.6)	20 (1.8)	33[c] (2.0)
Alveolar epithelium, hyperplasia, atypical	0	0	3 (3.7)	5[b] (3.2)
Metaplasia, squamous	0	1 (2.0)	8[c] (2.3)	3 (1.7)
Alveolar epithelium, metaplasia	2 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Inflammation, granulomatous	9 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Interstitium, fibrosis	7 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Proteinosis	0	36[c] (1.2)	49[c] (2.8)	49[c] (3.9)
Cyst	0	0	1 (4.0)	0
Alveolar/bronchiolar adenoma	0	1	10[c]	9[c]
Alveolar/bronchiolar carcinoma	0	2	6[b]	6[b]
Alveolar/bronchiolar adenoma or carcinoma	0	3	15[c]	15[c]
Squamous-cell carcinoma	0	0	1	1
Alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma or squamous-cell carcinoma	0	3	16[c]	16[c]

Table 13 (contd)

Lesions observed	No. of rats exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Adrenal medulla				
No. examined microscopically	48	49	50	48
Hyperplasia	8 (1.6)	7 (2.3)	11 (2.1)	13 (2.0)
Benign pheochromocytoma	2	1	3	8[b]
Benign, complex, or malignant pheochromocytoma	2	1	4	10[b]

From National Toxicology Program (1998)
[a] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked
[b] Significantly different ($p \leq 0.05$) from the chamber control group by the logistic regression test
[c] Significantly different ($p \leq 0.01$) from the chamber control group by the logistic regression test

3.2 Intratracheal instillation

Rat

Steinhoff and Mohr (1991) reported on the exposure of rats to a cobalt–aluminium–chromium spinel (a blue powder [purity unspecified], with the empirical formula Co[II] 0.66, Al 0.7, Cr[III] 0.3, O 3.66, made of a mixture of CoO, $Al(OH)_3$ and Cr_2O_3 ignited at 1250 °C; 80% of particles < 1.5 μm). Groups of 50 male and 50 female Sprague-Dawley rats, 10 weeks of age, received intratracheal instillations of 10 mg/kg bw of the spinel in saline every 2 weeks for 18 treatments (then every 4 weeks from the 19th to the 30th treatment) for 2 years. Control groups of 50 males and 50 females received instillations of saline only and other control groups of 50 males and 50 females remained untreated. Animals were allowed to live until natural death or were killed when moribund. No appreciable difference in body weights or survival times was observed between the treated and control groups [exact survival data not given]. Alveolar/bronchiolar proliferation was observed in 0/100 untreated controls, 0/100 saline controls, and in 61/100 rats treated with the spinel. [The Working Group noted that the nature of the bronchoalveolar proliferation or possible association with inflammation was not described.] No pulmonary tumours were observed in 100 untreated or 100 saline controls. In the group that received the spinel, squamous-cell carcinoma was observed in one male rat and two females (Steinhoff & Mohr, 1991).

3.3 Intramuscular injection

Rat

In studies undertaken by Heath (1954, 1956), groups of 10 male and 10 female hooded rats, 2–3 months old, received a single intramuscular injection of 28 mg cobalt-metal powder (spectrographically pure, 400 mesh; 3.5 μm × 3.5 μm to 17 μm × 12 μm with large numbers of long narrow particles of the order of 10 μm × 4 μm) in 0.4 mL fowl serum into the thigh; a control group of 10 males and 10 females received fowl serum only. Average survival times were 71 weeks in treated males and 61 weeks in treated females; survival of controls was not specified. During the observation period of 122 weeks, 4/10 male and 5/10 female treated rats developed sarcomas (mostly rhabdomyosarcomas) at the injection site compared with 0/20 controls. A further group of 10 female rats received a single intramuscular injection of 28 mg cobalt-metal powder in 0.4 mL fowl serum; others received injections of 28 mg zinc powder (five rats) or 28 mg tungsten powder (five rats). Average survival time for cobalt-treated rats was 43 weeks. During the observation period of 105 weeks, sarcomas (mostly rhabdomyosarcomas) developed in 8/10 cobalt powder-treated rats; none occurred in the zinc powder- or tungsten powder-treated rats. No other tumours occurred in any of the cobalt-treated or other rats, except for one malignant lymphoma in a zinc-treated rat (Heath, 1954, 1956). [The Working Group noted the small number of animals and questioned the relevance of the route of administration.]

In a supplementary study, a group of 30 male hooded rats, 2–3 months of age, received a single intramuscular injection of 28 mg cobalt-metal powder (spectrographically pure [particle size unspecified]) in 0.4 mL fowl serum into the right thigh; a control group of 15 males received a single injection of fowl serum only. The rats were killed at daily intervals 1 to 28 days after injection. An extensive and continuing breakdown of the differentiated muscle fibres into free myoblasts, and the transformation of some of these myoblasts were described (Heath, 1960). [The Working Group questioned the relevance of the route of administration.]

In a series of three experiments, each of 80 female hooded rats, 7–9 weeks of age, received intramuscular injections of 28 mg of 'wear' particles obtained by grinding continuously artificial hip or knee prostheses in Ringer's solution or synovial fluid in conditions simulating those occurring in the body. Prostheses were made of cobalt–chromium–molybdenum alloy (66.5% cobalt, 26.0% chromium, 6.65% molybdenum, 1.12% manganese). Particles (diameter, down to 0.1 μm [mostly 0.1–1 μm]) were injected in 0.4 mL horse serum and the rats were observed for up to 29 months [survival not specified]. No control group was reported. Sarcomas developed at the injection site in 3/16, 4/14 and 16/50 rats in the three series, respectively. Approximately half of the tumours were rhabdomyosarcomas; the remainder were mostly fibrosarcomas (Heath *et al.*, 1971; Swanson *et al.*, 1973).

3.4 Intramuscular implantation

3.4.1 *Rat*

As a follow-up to the studies by Heath *et al.* (1971) and Swanson *et al.* (1973) (see above), groups of female Wistar and hooded rats, weighing 190–310 and 175–220 g, respectively, received intramuscular implants of 28 mg coarse (100–250 μm diameter; 51 Wistar rats) or fine (0.5–50 μm diameter, 85% 0.5–5 μm; 61 Wistar and 53 hooded rats) particles of a dry powder, obtained by grinding a cobalt–chromium–molybdenum alloy (68% cobalt, 28% chromium, 4% molybdenum). The animals were observed for life. A sham-operated control group of 50 female Wistar rats was included. Survival at 2 years was: 11/51 rats receiving the coarse particles, 7/61 Wistar rats receiving the fine particles, 0/53 hooded rats receiving the fine particles and 5/50 Wistar controls. No tumour was noted at the implantation site of rats treated with either coarse or fine alloy particles nor in sham-operated control animals (Meachim *et al.*, 1982).

3.4.2 *Guinea-pig*

In a similar study in guinea-pigs (Meachim *et al.*, 1982), a group of 46 female Dunkin-Hartley guinea-pigs, weighing 550–930 g, received intramuscular implants of 28 mg powdered cobalt–chromium–molybdenum alloy (68% cobalt, 28% chromium, 4% molybdenum; particle diameter, 0.5–50 μm) and were observed for life; 12/46 animals were alive at 3 years. No control group was reported. No tumours were observed at the implantation site; nodular fibroblastic hyperplasia was observed at the implantation site in eight animals (Meachim *et al.*, 1982).

3.5 Subcutaneous implantation

Rat

Groups of five male and five female Wistar rats, 4–6 weeks of age, received subcutaneous implants of one pellet (approximately 2 mm in diameter) of a cobalt–chromium–molybdenum (Vitallium) alloy. The percentage composition of the metal constituents of the Vitallium alloy was not given. Animals were observed for up to 27 months [survival of animals receiving cobalt–chromium–molybdenum alloy not given]. No sarcomas developed in rats that received the pellets (Mitchell *et al.*, 1960).

3.6 Intra-osseous implantation

3.6.1 *Rat*

Groups of 10–17 male and 8–15 female Sprague-Dawley rats, 30–43 days of age, received implants of one of seven test materials containing cobalt alloyed with chromium and nickel, molybdenum, tungsten and/or zirconium, with traces of other elements (as small

rods, 1.6 mm diameter and 4 mm length, powders or porous compacted wire), in the femoral bone and were observed for up to 30 months. Groups of 13 male and 13 female untreated and sham-operated controls were available. Average survival time was more than 21 months. Sarcomas at the implant site were observed in 1/18 rats given cobalt-based alloy powder containing 41% cobalt, 3/26 rats (given a nickel–cobalt-based powder containing 33% cobalt and 3/32 rats given porous compacted wire containing 51% cobalt. No tumours were observed in two groups of 25 rats given rods containing 69 or 47% cobalt, in two groups of 26 rats given rods containing 0.11 or 33% cobalt, in two groups of 25 and 26 untreated rats nor in a group of 26 sham-treated control rats (Memoli *et al.*, 1986).

3.6.2 *Rabbit*

Two groups of 15–20 rabbits [strain, sex and age unspecified] received an implantation in the femoral cavity of metallic chromium dust or metallic cobalt dust [purity and particle size unspecified]. Physical examination by palpation and X-ray examination 3 years after implantation revealed no implantation-site tumour in survivors of the chromium-treated group nor in the six survivors of the cobalt-treated group (Vollman, 1938). In a follow-up study of survivors [number unspecified] at intervals up to 6 years after implantation, sarcomas were observed at the implantation site in three chromium-treated and two cobalt-treated rabbits (Schinz & Uehlinger, 1942). [The Working Group noted the limited reporting.]

3.7 Intraperitoneal injection

Rat

Groups of 10 male and 10 female Sprague-Dawley rats, 10 weeks of age, received three intraperitoneal injections at 2-month intervals of saline or cobalt–aluminium–chromium spinel powder (see Section 3.2) in saline (total dose, 600 mg/kg bw). Animals were allowed to live their natural lifespan or were sacrificed when moribund [survival not given]. Malignant peritoneal tumours occurred in 1/20 controls (histiocytoma) and 2/20 spinel-treated animals (one histiocytoma, one sarcoma) (Steinhoff & Mohr, 1991).

3.8 Intrarenal administration

Rat

Groups of female Sprague-Dawley rats, weighing 120–140 g, received a single injection of 5 mg metallic cobalt powder (20 rats) or cobalt sulfide powder (18 rats) [purity and particle size unspecified] suspended in 0.05 mL glycerine into each pole of the right kidney. A control group of 16 female rats received injections of 0.05 mL glycerine into each pole of the kidney. After 12 months, necropsies were performed on all rats; no tumours were observed in the kidneys of treated or control rats (Jasmin & Riopelle, 1976). [The Working

Group noted the short duration and inadequate reporting of the experiment and the unusual site of administration.]

3.9 Intrathoracic injection

Rat

Two groups of 10 female hooded rats, 2–3 months of age, received intrathoracic injections of 28 mg cobalt-metal powder (spectrographically pure; particle size, < 400 mesh; 3.5 μm × 3.5 μm to 17 μm × 12 μm, with many long narrow particles of the order of 10 μm × 4 μm) in serum [species unspecified] through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group) and were observed for up to 28 months. Death occurred within 3 days of the treatment in 6/10 rats injected through the diaphragm and in 2/10 rats injected through the intercostal space. The remaining rats in the first group (diaphragm) survived 11–28 months and in the second group (intercostal space), 7.5–17.5 months. Of the 12 rats that survived the injection, four developed intrathoracic sarcomas (three of mixed origin, including rhabdomyosarcomatous elements; one rhabdomyosarcoma arising in the intercostal muscles) (Heath & Daniel, 1962). [The Working Group noted the small numbers of animals and the questionable route of administration.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Deposition, retention, clearance and metabolism

Several reviews of the toxicology of cobalt, including toxicokinetic aspects, are available (IARC, 1991; Midtgård & Binderup, 1994; Lauwerys & Lison, 1994; Lison, 1996; Barceloux, 1999). This section will focus on the toxicokinetic data published since the previous IARC evaluation (1991) and potentially relevant for cancer. Particular emphasis will be put on studies that examined the fate of inhaled hard-metal particles and related components, when available.

Solubilization of cobalt from tungsten carbide–cobalt powder

It has been shown that tungsten carbide–cobalt powder (WC–Co) is more toxic to murine macrophages *in vitro* than pure cobalt-metal particles, and that the cellular uptake of cobalt is enhanced when the metal is present in the form of WC–Co (Lison & Lauwerys, 1990). In a further study by the same authors, the solubilization of cobalt in the extracellular milieu was shown to increase in the presence of WC. This phenomenon, however, does not explain the greater toxicity of the WC–Co mixture, because increasing the amount of solubilized cobalt in the extracellular medium in the absence of WC did not

result in increased toxicity. Moreover, the amount of cobalt solubilized from a toxic dose of WC–Co was insufficient alone to affect macrophage viability. A toxic effect was only observed when the WC–Co mixture came into direct contact with the cells. These results indicate that the toxicity of the WC–Co mixture does not result simply from an enhanced bioavailability of its cobalt component and suggest that hard-metal dust behaves as a specific toxic entity (Lison & Lauwerys, 1992).

4.1.1 *Humans*

(*a*) *Deposition and retention*

Since the previous IARC (1991) evaluation, no additional relevant data concerning the deposition and/or retention of inhaled cobalt-containing particles in humans have been located.

In several studies conducted on lung tissue or bronchoalveolar lavage fluid (BALF) from patients with lung disease induced by hard-metal particles (hard-metal disease), the presence of tungsten, tantalum or titanium particles was detected, but no or insignificant amounts of cobalt were found (Lison, 1996).

Citizens in Catalonia, Spain, were found to have cobalt in their lungs at the limit of detection (Garcia *et al.*, 2001). In contrast, citizens of Mexico City showed remarkably high concentrations of cobalt in their lungs over three decades, which was attributed to air pollution (Fortoul *et al.*, 1996). In an autopsy study carried out in Japan, cobalt concentrations in the lung were reported to be related mainly to blood concentrations and were found to be lower in patients who had died from lung cancer than from other causes (Adachi *et al.*, 1991; Takemoto *et al.*, 1991). A study of uranium miners in Germany demonstrated by NAA that cobalt, associated with uranium, arsenic, chromium and antimony was present at high concentrations in the lungs, with or without concurrent lung tumours, even 20 years after cessation of mining (Wiethege *et al.*, 1999).

(*b*) *Intake and absorption*

There are few data on the respiratory absorption of inhaled cobalt-containing materials in humans. The absorption rate is probably dependent on the solubility in biological fluids and in alveolar macrophages of the cobalt compounds under consideration. Increased excretion of the element in post-shift urine of workers exposed to soluble cobalt-containing particles (cobalt metal and salts, hard-metal particles) has been interpreted as an indirect indication of rapid absorption in the lung; in contrast, when workers were exposed to the less soluble cobalt oxide particles, the pattern of urinary excretion indicated a lower absorption rate and probably a longer retention time in the lung (Lison & Lauwerys, 1994; Lison *et al.*, 1994). The importance of speciation and solubility for respiratory absorption has also been highlighted by Christensen and Mikkelsen (1986). These authors found that cobalt concentrations in blood and urine increased (0.2–24 μg/L and 0.4–848 μg/L, respectively) in pottery plate painters using a soluble cobalt paint compared to the control group of painters without cobalt exposure (0.05–0.6 μg/L and 0.05–7.7 μg/L, respectively). The

pottery painters exposed to slightly soluble cobalt paint had only slightly increased cobalt concentrations compared to controls (see Section 1.3.2(*d*)).

The absorption of cobalt compounds has been estimated to vary from 5 to 45% of an orally-administered dose (Valberg *et al.*, 1969; Smith *et al.*, 1972; Elinder & Friberg, 1986). The mean urinary excretion within 24 h of radioactive cobalt (from cobalt chloride) given orally at 20 μmoles to 17 volunteers was estimated to be about 18% (Sorbie *et al.*, 1971). In a short-term cross-over study in volunteers, the gastrointestinal uptake of soluble cobalt chloride measured as cobalt concentrations in urine was found to be considerably higher than that of insoluble cobalt oxide (urine ranges, < 0.17–4373 and < 0.17–14.6 nmol/mmol creatinine, respectively). It was also shown that ingestion of controlled amounts of soluble cobalt compounds resulted in significantly higher cobalt concentrations in urine ($p < 0.01$) in women (median, 109.7 nmol/mmol creatinine) than in men (median, 38.4 nmol/mmol creatinine), suggesting that the gastrointestinal uptake of cobalt is higher in women than men (Christensen *et al.*, 1993).

Cobalt has been detected in pubic hair, toe nails and sperm of some but not all workers diagnosed with hard-metal disease (Rizzato *et al.*, 1992, 1994; Sabbioni *et al.*, 1994a).

It was found that absorption of cobalt through the skin and gastrointestinal tract also contributed to concentrations of cobalt in urine in occupationally-exposed individuals (Christensen *et al.*, 1993; Scansetti *et al.*, 1994; Christensen, 1995; Linnainmaa & Kiilunen, 1997). Concentrations of cobalt in urine of smokers at a hard-metal factory were higher than those in nonsmokers (10.2 nmol/L [0.6 μg/L] versus 5.1 nmol/L [0.3 μg/L] on average), while no difference in concentrations of cobalt in blood was detected (Alexandersson, 1988). However, the cobalt excreted in urine was found not to be derived from cobalt contained in cigarettes nor from daily intake of vitamin B_{12}, but through eating and smoking with cobalt-contaminated hands at work (Linnainmaa & Kiilunen, 1997).

After absorption, cobalt is distributed systemically but does not accumulate in any specific organ, except the lung in the case of inhalation of insoluble particles. Normal cobalt concentrations in human lung have been reported to be 0.27 ± 0.40 (mean ± SD) μg/g dried lung based on tissue samples taken from 2274 autopsies in Japan (Takemoto *et al.*, 1991). A majority of the autopsies were carried out on subjects with malignant neoplasms. There was no increase in cobalt concentration with age, no gender difference and no association with degree of emphysema nor degree of contamination (the grade of particle deposition in the lung).

The normal concentration of cobalt in blood is in the range of 0.1–0.5 μg/L and that in urine is below 2 μg/L in non-occupationally exposed persons. The concentrations of cobalt in blood, and particularly in urine, increase in proportion to the degree of occupational (inhalation) exposure and may be used for biological monitoring in order to assess individual exposure (Elinder & Friberg, 1986). As well as the high concentrations found in workers exposed to cobalt, increased concentrations of cobalt have been found in blood (serum) of uraemic patients (Curtis *et al.*, 1976; Lins & Pehrsson, 1976) and in urine of individuals taking multivitamin preparations (as cyanocobalamin, a source of cobalt) (Reynolds, 1989) (see also IARC, 1991 and Section 1.1.5).

(*c*) *Excretion*

The major proportion of systemically-distributed cobalt is cleared rapidly (within days) from the body, mainly via urine, but a certain proportion (10%) has a longer biological half-life, in the range of 2–15 years (Newton & Rundo, 1970; Elinder & Friberg 1986). Of an oral dose of cobaltous chloride, 6–8% was eliminated within 1 week in normal healthy persons (Curtis *et al.*, 1976). The elimination of cobalt is considerably slower in patients undergoing haemodialysis, which supports the importance of renal clearance (Curtis *et al.*, 1976). In workers in the hard-metal industry, it has been shown that concentrations of cobalt in urine increase rapidly in the hours that follow cessation of exposure, with a peak of elimination about 2–4 h after exposure, and a subsequent decrease (more rapid in the first 24 h) in the following days (Apostoli *et al.*, 1994).

4.1.2 *Experimental systems*

Following subcutaneous administration of cobalt chloride (250 μmol/kg bw) to rats, cobalt was found predominantly (> 95%) in plasma, from which it was rapidly eliminated (half-life ($t_{1/2}$), approximately 25 h) (Rosenberg, 1993). In-vitro studies (Merritt *et al.*, 1984) have shown that cobalt ions bind strongly to circulating proteins, mainly albumin. Edel *et al.* (1990) reported the in-vitro interaction of hard metals with human lung and plasma components and identified three biochemical pools of cobalt with different molecular weights in the lung cytosol. It has been suggested that cobalt binding to proteins may be of significance for immunological reactions involving cobalt as a hapten (Sjögren *et al.*, 1980). Wetterhahn (1981) showed that oxyanions of chromium, vanadium, arsenic and tungsten enter cells using the normal active transport system for phosphate and sulfate and may inhibit enzymes involved in phosphoryl or sulfuryl transfert reactions. Similarly, the divalent ions of cobalt may complex small molecules such as enzymes and alter their normal activity.

While cobalt-metal particles are practically insoluble in water, the solubilization of these particles is greatly enhanced in biological fluids due to extensive binding to proteins (0.003 mg/L in physiological saline, but 152.5 mg/L in human plasma at 37 °C) (Harding, 1950) and is increased up to sevenfold in the presence of WC particles (in oxygenated phosphate buffer at 37 °C) (Lison *et al.*, 1995).

(*a*) *In-vivo studies*

Gastrointestinal absorption of cobalt in rats is dependent on the dose, the ratio of iron to cobalt and the status of body iron stores (Schade *et al.*, 1970). It has been shown that following oral administration of cobalt chloride, 75% is eliminated in faeces and the highest accumulation of cobalt is found in liver, kidney, heart and spleen (Domingo *et al.*, 1984; Domingo, 1989; Ayala-Fierro *et al.*, 1999).

Following intravenous administration of cobalt chloride to rats, 10% of the dose was found to be excreted in faeces, indicating that cobalt can be secreted in the bile. Elimination was triphasic. During the first 4 h, cobalt was rapidly cleared from blood with a

half-life of 1.3 h. The second phase from 4 h to 12 h demonstrated a slower clearance rate with a half-life of 4.3 h. The final phase from 12 h to 36 h had a half-life of 19 h (Ayalu-Fierro *et al.*, 1999).

Kyono *et al.* (1992) exposed rats to ultrafine metallic cobalt particles (mean primary diameter, 20 nm) using a nebulizer producing droplets (MMAD, 0.76 μm; GSD, 2.1; concentration, 2.12 ± 0.55 mg/m^3) for 5 h per day for 4 days and induced reversible lung lesions. Clearance from the lung followed two phases: 75% of the cobalt was cleared within 3 days with a biological half-life of 53 h; the second phase from 3 days to 28 days had a slower clearance rate with a half-life of 156 h.

Kreyling *et al.* (1993) performed clearance studies using inhalation of monodisperse, porous cobalt oxide particles (MMAD, 1.4 and 2.7 μm) in Long-Evans rats. Of the small and large particles, 37% and 38%, respectively, were eliminated in the faeces within 3 days. The half-life for long-term thoracic retention was 25 and 53 days, respectively. After 6 months, large and small cobalt particles were still distributed in the bodies of the rats, mainly in the lung (91 and 52%), skeleton (6 and 22%) and in soft tissue (1.4 and 17%), respectively.

Lison and Lauwerys (1994) found that when non-toxic doses of cobalt metal were administered intratracheally to rats either alone (0.03 mg/100 g body weight) or mixed with tungsten carbide (0.5 mg/100 g body weight; WC–Co containing 6% of cobalt-metal particles), the retention time of the metal in the lung was longer in cobalt- than in WC–Co-treated animals. After 1 day, the lungs of animals instilled with cobalt alone contained twice as much cobalt as in those administered the same amount of cobalt as WC–Co (12 versus 5 μg cobalt/g lung after 24 h).

Slauson *et al.* (1989) induced patchy alveolitis, bronchiolitis and inflammation in the lungs of calves using parainfluenza-3 virus followed by a single inhalation exposure to an aerosol of submicronic cobalt oxide (total dose, about 80 mg). The virus-exposed calves retained 90% of initial cobalt lung burden at day 7 compared with 51% retention in controls. This difference was still present at day 21. Pneumonic calves also exhibited decreased translocation of particles to regional lymph nodes. The authors suggested impaired particulate clearance from acutely-inflamed lungs, which implicated decreased mucociliary clearance and interstitial sequestration within pulmonary alveolar macrophages as the major contributing factors.

(*b*) *In-vitro studies*

In-vitro dissolution of monodisperse, 2.7-μm cobalt oxide particles in baboon alveolar macrophage cell cultures was found to be three times higher than in a cell-free system; the daily dissolution rate was 0.25% versus 0.07% and 0.09% for beads containing particles only and particles combined with alveolar macrophages, respectively (Lirsac *et al.*, 1989). Kreyling *et al.* (1990) studied in-vitro dissolution of cobalt oxide particles in human and canine alveolar macrophages and found that smaller particles had faster dissolution rates. In-vitro dissolution rates were found to be similar to in-vivo translocation rates previously

found for human and canine lung. Dissolution of ultrafine cobalt powder in artificial lung fluid was six times higher than that of standard cobalt powder (Kyono *et al.*, 1992).

Collier *et al.* (1992) studied factors influencing in-vitro dissolution rates in a simple non-cellular system using 1.7 μm count median diameter (CMD) porous cobalt oxide particles and cobalt-labelled fused aluminosilicate (Co-FAP). Less than 0.5% of cobalt oxide and 1.8% of Co-FAP dissolved over 3 months. The difference in dissolution was much greater in the first week than in the following weeks, with Co-FAP being 20 times more soluble. The dissolution rate for cobalt oxide was higher at lower pH. Lundborg *et al.* (1992) measured and changed phagolysosomal pH within rabbit alveolar macrophages. No clear effect on cobalt dissolution rate was detected for 0.6-μm cobalt oxide particles at pH values ranging between 5.1 and 5.6. Lundborg *et al.* (1995) also studied the effect of phagolysosomal size on cobalt dissolution in rabbit alveolar macrophages incubated with sucrose and in human alveolar macrophages from smokers and non-smokers. The authors found no difference in cobalt dissolution in either rabbit or human cells in spite of large differences in morphological appearance of the macrophages.

Lison and Lauwerys (1994) found that cellular uptake of cobalt was greater when the metal was presented to mouse macrophages as WC–Co. This increased bioavailability of cobalt from hard-metal particles has been interpreted as the result of a physicochemical interaction between cobalt metal and tungsten carbide particles (Lison *et al.*, 1995).

In-vitro exposure of HeLa (tumour) cells to cobalt chloride has been shown to result in the intracellular accumulation of cobalt (Hartwig *et al.*, 1990).

4.2 Toxic effects

4.2.1 *Humans*

The health effects resulting from exposure to metallic cobalt-containing particles may be subdivided into local and systemic effects. Local effects are those that occur at the points of contact or deposition of the particles, the skin and the respiratory tract; these effects may be due to the particles themselves (as a result of surface interactions between the particles and biological targets) and/or to cobalt ions solubilized from the particles. Toxic effects outside the respiratory tract are unlikely to be caused by the metallic particles themselves, but result from the release of cobalt ions from the particles and their subsequent absorption into the circulation. (Systemic effects may also be indirect consequences of the damage caused in the lungs).

(*a*) *Dermal effects*

The skin sensitizing properties of cobalt are well known, both from human experience and from animal testing (Veien & Svejgaard, 1978; Wahlberg & Boman, 1978; Fischer & Rystedt, 1983). Exposure to cobalt may lead to allergic contact dermatitis, sometimes having features of an airborne dermatitis, particularly in hard-metal workers (Dooms-Goossens *et al.*, 1986). Urticarial reactions have also been described. Cross-reaction with

nickel (as well as co-sensitization) is frequent (Shirakawa *et al.*, 1990). The dermal effects of cobalt may occur with all forms of cobalt, i.e. cobalt metal and other cobalt compounds, such as salts.

(*b*) *Respiratory effects*

The various respiratory disorders caused by the inhalation of metallic cobalt-containing particles have been extensively reviewed (Balmes, 1987; Cugell *et al.*, 1990; Seghizzi *et al.*, 1994; Lison, 1996; Barceloux, 1999; Nemery *et al.*, 2001a). These particles may cause non-specific mucosal irritation of the upper and lower airways leading to rhinitis, sinusitis, pharyngitis, tracheitis or bronchitis, but the main diseases of concern are bronchial asthma and a fibrosing alveolitis known as hard-metal lung disease.

(i) *Bronchial asthma*

Bronchial asthma, which like contact dermatitis is presumably based on immunological sensitization to cobalt, has been described in workers exposed to various forms of cobalt, i.e. not only in workers exposed to hard-metal dust, but also in those exposed to 'pure' cobalt particles (Swennen *et al.*, 1993; Linna *et al.*, 2003), as well as in subjects exposed to other cobalt compounds, such as cobalt salts. Occupational asthma is more frequent than fibrosing alveolitis in hard-metal workers or workers exposed to cobalt dust, but occasionally the two conditions co-exist (Davison *et al.*, 1983; Van Cutsem *et al.*, 1987; Cugell *et al.*, 1990). Chronic bronchitis is reported to be quite prevalent in hard-metal workers, particularly in older studies when dust exposure was considerable and smoking status was not well ascertained (Tolot *et al.*, 1970). It is not clear whether those patients with airway changes (asthma or chronic obstructive lung disease) represent 'airway variants' of the same respiratory disease, or whether the pathogenesis of these airway changes is altogether different from that of parenchymal changes. Earlier autopsy studies frequently indicated the presence of emphysema in patients with hard-metal lung disease.

(ii) *Hard-metal lung disease*

Interstitial (or parenchymal) lung disease caused by metallic cobalt-containing particles is a rare occupational lung disease. Several reviews are available on this fibrosing alveolitis which is generally called hard-metal lung disease (Bech *et al.*, 1962; Anthoine *et al.*, 1982; Hartung, 1986; Balmes, 1987; Van Den Eeckhout *et al.*, 1988; Cugell, 1992; Seghizzi *et al.*, 1994; Lison, 1996; Newman *et al.*, 1998; Nemery *et al.*, 2001a,b). A discussion of the occurrence and features of interstitial lung disease caused by metallic cobalt-containing compounds is not only relevant in itself, but it may also have a bearing on the risk of lung cancer, because fibrosing alveolitis and lung cancer may be related mechanistically with regard to both oxidative damage and inflammatory events. Moreover, there is some evidence from observations in humans that lung fibrosis represents a risk for lung cancer, although this evidence is not unequivocal (Bouros *et al.*, 2002).

Terminology of (interstitial) hard-metal lung disease

The terminology used to label this disease is complex and confusing. Especially in the earlier literature, hard-metal disease was mostly referred to as a pneumoconiosis (e.g. hard-metal pneumoconiosis or tungsten carbide pneumoconiosis). This term is justified inasmuch as pneumoconiosis is defined as "the non-neoplastic reaction of the lungs to inhaled mineral or organic dust and the resultant alteration in their structure, but excluding asthma, bronchitis and emphysema" (Parkes, 1994). However, it can be argued that the term pneumoconiosis is not entirely appropriate, because it suggests that the disease results from the accumulation of high quantities of dust in the lungs and this is not always the case in hard-metal workers. Indeed, like hypersensitivity pneumonitis and chronic beryllium disease, hard-metal lung disease differs from the common mineral pneumoconioses in that the occurrence of the disease is not clearly related to the cumulative dust burden, but is more probably due to individual susceptibility. Thus the term hard-metal pneumoconiosis has tended to be abandoned in favour of 'hard-metal lung disease'. An advantage, but also a drawback, of the latter term is that the respiratory effects of exposure to hard-metal dust include not only interstitial lung disease (pneumonitis, fibrosis), but also (and probably more frequently) airway disorders, such as bronchitis and occupational asthma. Therefore, the phrases 'hard-metal lung', 'hard-metal disease' or 'hard-metal lung disease' usually encompass more than just the parenchymal form of the disease (Nemery *et al.*, 2001a).

In its most typical pathological presentation, this interstitial lung disease consists of a giant-cell interstitial pneumonia (GIP), one of the five types of interstitial pneumonias originally described by Liebow (1975). GIP is now accepted as being pathognomonic of hard-metal lung disease. Ohori *et al.* (1989) reviewed the published literature and concluded that GIP is indeed highly specific for hard-metal lung, since they found only three published cases of GIP that had not had exposure to cobalt or hard metal. However, while there is no doubt that GIP should be considered to be hard-metal lung disease unless proven otherwise, not all patients with hard-metal lung disease have a 'textbook presentation' of GIP. Indeed, the lung pathology in hard-metal lung disease is variable depending on, among other factors, the stage of the disease and probably also on its pathogenesis in individual patients. The pathology in some patients may be more reminiscent of mixed dust pneumoconiosis (Bech, 1974). Moreover, a pathological diagnosis is not always available.

The most compelling argument against the term hard-metal lung disease is that the disease may also occur without exposure to hard-metal dust. This was established when GIP was found in diamond polishers in Belgium shortly after the introduction of a new technology to facet diamonds. These workers were exposed not to hard-metal dust, but to cobalt-containing dust that originated from the use of high-speed cobalt–diamond polishing discs (Demedts *et al.*, 1984). This observation confirmed an earlier hypothesis that cobalt, rather than tungsten carbide, is responsible for hard-metal lung, and it led to the proposal that the interstitial lung disease should be called 'cobalt pneumopathy' or 'cobalt lung', rather than hard-metal lung (Lahaye *et al.*, 1984). Nevertheless, the term cobalt lung is not entirely appropriate either, because not all types of exposure to cobalt appear to lead to interstitial lung disease.

Table 14 summarizes the various terms which have been used for the interstitial lung disease caused by hard-metal and cobalt dust. The most correct term is probably 'cobalt-related interstitial lung disease', but this would add more confusion and thus the term hard-metal lung disease will be used here.

Table 14. Terminology of hard-metal lung disease

Name of disease (term)	Features supporting use of term	Features not supporting use of term	Reference
Hard-metal pneumoconiosis	Lung disease is caused by exposure to hard-metal dust	Pathogenesis (hypersensitivity) differs from that of mineral pneumoconiosis.	Parkes (1994)
Tungsten carbide pneumoconiosis	Tungsten carbide is main component of hard metal	Tungsten carbide is not the actual causative agent.	
Hard-metal disease Hard-metal lung Hard-metal lung disease	Lung disease is caused by exposure to hard metal	Terms encompass interstitial lung disease as well as airway disease, such as asthma; similar disease may be caused by exposure to materials other than hard metal.	Nemery *et al.* (2001a)
Cobalt lung or cobalt pneumopathy	Cobalt is the most critical toxic agent	Not all types of exposure to cobalt lead to interstitial lung disease.	Lahaye *et al.* (1984)
Giant cell interstitial pneumonia (GIP)	Pathognomonic pathological feature	Pathology not always available and not always present in individual cases	Ohori *et al.* (1989)

Adapted from Nemery *et al.* (2001a)

Pathogenesis of hard-metal lung disease

There is little doubt that cobalt plays a critical role in the pathogenesis of hard-metal lung disease. Studies in experimental systems have demonstrated that WC–Co particles exhibit a unique pulmonary toxicity compared with cobalt particles (see Section 4.2.2). The toxicity is probably due, at least in part, to the production of toxic oxygen species. However, the hard-metal lung disease that occurs in humans has never been reproduced in experimental animals; neither the typical pattern of inflammation (GIP), nor the progressive nature of the fibrosis.

The basis of individual susceptibility to develop hard-metal lung disease is not known. Cobalt is known to elicit allergic reactions in the skin, probably via cell-mediated pathways (Veien & Svejgaard, 1978), but the relationship, if any, between this cell-mediated allergy and GIP is unknown. Occasionally, patients have been found to have both cobalt dermatitis and interstitial lung disease (Sjögren *et al.*, 1980; Cassina *et al.*, 1987; Demedts & Ceuppens, 1989). Immunological studies (Shirakawa *et al.*, 1988; Kusaka *et al.*, 1989;

Shirakawa *et al.*, 1989, 1990; Kusaka *et al.*, 1991; Shirakawa *et al.*, 1992) have found both specific antibodies and positive lymphocyte transformation tests against cobalt (as well as nickel) in some patients with hard-metal asthma. However, to date, the immunopathogenesis of GIP is unknown. In one case of GIP, expression of intracellular transforming growth factor β1 (TGF-β1) was shown in alveolar macrophages, including multinucleate forms, and in hyperplastic alveolar epithelium (Corrin *et al.*, 1994). In another case of GIP, immunolocalization of tumour necrosis factor α (TNF-α) was found to be highly associated with the infiltrating mononuclear cells within the interstitium and with cannibalistic multinucleated giant cells in the alveolar spaces (Rolfe *et al.*, 1992). The involvement of autoimmune processes is suggested by the report of the recurrence of GIP in a transplanted lung (Frost *et al.*, 1993) and in one case of hard-metal alveolitis accompanied by rheumatoid arthritis (Hahtola *et al.*, 2000).

Recent evidence also indicates that the susceptibility to develop cobalt-related interstitial (hard-metal) lung disease is associated with the HLA-DPB1*02 allele, i.e. with the presence of glutamate at position 69 in the HLA-DPB chain (Potolicchio *et al.*, 1997), probably because of a high affinity of the HLA-DP molecule for cobalt (Potolicchio *et al.*, 1999). It should be noted that the HLA-DPB1*02 allele is the same as that associated with susceptibility to chronic beryllium disease (Richeldi *et al.*, 1993).

Clinical presentation

The clinical presentation of hard-metal lung disease is variable: some patients present with subacute alveolitis and others with chronic interstitial fibrosis (Balmes, 1987; Cugell *et al.*, 1990; Cugell, 1992). In this respect, hard-metal lung disease is somewhat similar to hypersensitivity pneumonitis (extrinsic allergic alveolitis). Thus, the patient may experience work-related bouts of acute illness, which may lead progressively to pronounced disease with more persistent shortness of breath; but in other instances, the course of the disease is more insidious and the work-relatedness of the condition is not clearly apparent.

Most studies have found no relation between disease occurrence and length of occupational exposure. Subacute presentations may be found in young workers after only a few years exposure, but may also occur in older workers with very long careers. Chronic presentations are more likely in older subjects. The role of smoking in the susceptibility to hard-metal disease has not been evaluated thoroughly, but it is possible that non-smokers are slightly over-represented (Nemery *et al.*, 2001a).

Epidemiology

Descriptions of the epidemiology of hard-metal lung disease can be found in Lison (1996) and Newman *et al.* (1998). Precise incidence figures are not available. Clinical surveys and cross-sectional studies in the hard-metal industry have shown that typical hard-metal lung disease is a relatively rare occurrence, affecting a small percentage of the workforce at most (Miller *et al.*, 1953; Bech *et al.*, 1962; Dorsit *et al.*, 1970; Coates & Watson, 1971; Sprince *et al.*, 1984; Kusaka *et al.*, 1986; Sprince *et al.*, 1988; Meyer-Bisch *et al.*, 1989; Tan *et al.*, 2000), unless conditions of hygiene are very poor (Auchincloss

et al., 1992; Fischbein *et al.*, 1992). In diamond polishers in Belgium, the prevalence of cobalt-related occupational respiratory disease, including both airway and interstitial lung disease, has been estimated at about 1% of the total workforce (Van den Eeckhout *et al.*, 1988). A cross-sectional survey of 10 workshops, involving a total of 194 polishers, found no cases of overt lung disease, but there was a significant inverse relationship between spirometric indices of pulmonary function and mean levels of exposure to cobalt as assessed by ambient air or biological monitoring (Nemery *et al.*, 1992).

Lung disease has been associated not only with the manufacture of cobalt–diamond tools (Migliori *et al.*, 1994), but also with their use, at least in the case of high-speed cobalt–diamond discs used for diamond polishing (Demedts *et al.*, 1984; Lahaye *et al.*, 1984; Wilk-Rivard & Szeinuk, 2001; Harding, 2003). This could be explained by the fact that the projection of cobalt in bonded diamond tools is higher (up to 90%) than in hard metal.

Carbide coatings can now also be deposited by flame or plasma guns onto softer substrates to harden their surfaces, and this process also exposes workers to a risk of hard-metal lung disease (Rochat *et al.*, 1987; Figueroa *et al.*, 1992).

A detailed and comprehensive cross-sectional survey of 82 workers exposed to cobalt compounds in a plant in Belgium involved in cobalt refining and 82 sex- and age-matched controls from the same plant found no radiological or functional evidence of interstitial lung disease in spite of substantial exposure to cobalt (mean duration of exposure, 8 years; range, 0.3–39.4 years; mean cobalt concentration in air, 125 $\mu g/m^3$ with about a quarter of the workers having had exposures above 500 $\mu g/m^3$) and (subclinical) evidence for other effects of cobalt (thyroid metabolism and haematological parameters) (Swennen *et al.*, 1993). The absence of interstitial lung disease in workers exposed to cobalt-metal particles in the absence of other compounds such as tungsten carbide has recently been confirmed in another cross-sectional survey of 110 current and former cobalt refinery workers and 140 control workers in Finland (Linna *et al.*, 2003). These cross-sectional studies suggest (but do not prove) that exposure to even relatively high levels of cobalt-metal particles does not lead to interstitial lung disease (although such exposure does lead to asthma).

There is no published evidence for the occurrence of typical 'hard-metal lung disease' in workers exposed to cobalt-containing alloys, although adverse respiratory effects may be associated with the manufacture or maintenance of some cobalt-containing alloys (Deng *et al.*, 1991; Kennedy *et al.*, 1995). Dental technicians (who are exposed to a variety of agents, including cobalt) may also develop interstitial lung disease (Lob & Hugonnaud, 1977; De Vuyst *et al.*, 1986; Sherson *et al.*, 1988; Selden *et al.*, 1995).

Interstitial lung disease has not been described in workers exposed to cobalt salts, except for a study describing four cases of pulmonary fibrosis in a cobalt carbonate factory that operated before the Second World War (Reinl *et al.*, 1979).

It is conceivable that full-blown hard-metal lung represents a 'tip of the iceberg phenomenon' and that there is other less specific pulmonary damage in many more subjects. The relationship of overt or latent disease with exposure levels remains unknown. This is due, in

part, to the role of individual susceptibility factors, but also to the nature of the hard-metal industry, which is often composed of relatively small tool manufacturing plants or repair workshops, thus making large and comprehensive surveys of the industry rather difficult. In addition, epidemiological studies of a rare and specific condition, such as hard-metal lung, are also difficult because of the poor sensitivity of conventional epidemiological techniques such as questionnaire studies, pulmonary function testing and chest X-ray. Moreover, cross-sectional studies are not the best method to detect clinical cases of hard-metal lung disease, because of the healthy worker effect, and possibly also because of a 'healthy workshop effect' (Nemery *et al.*, 1992). The latter refers to the frequently-experienced fact that the factories with the poorest occupational hygiene practice, and therefore probably those with the highest attack rates, are also the least likely to participate in health surveys (Auchincloss *et al.*, 1992).

(*c*) *Extrapulmonary effects*

Cobalt exerts a number of toxic effects outside the respiratory system (IARC, 1991; Lison, 1996), which are not specific for metallic cobalt-containing particles. Cobalt stimulates erythropoiesis, thus possibly causing polycythaemia, and has been used in the past for the treatment of anaemia (Alexander, 1972; Curtis *et al.*, 1976). Cobalt is toxic to the thyroid (Kriss *et al.*, 1955; Little & Sunico, 1958) and it is cardiotoxic (see IARC, 1991). The occurrence of cardiomyopathy in occupationally-exposed workers has been investigated and there is some evidence that it may occur, although this is still debated (Horowitz *et al.*, 1988; Jarvis *et al.*,. 1992; Seghizzi *et al.*, 1994).

Possible neuropsychological sequelae, consisting of deficits in encoding or slowed memory consolidation, have been reported in patients with hard-metal disease (Jordan *et al.*, 1990, 1997).

4.2.2 *Experimental systems*

Cobalt and its various compounds and/or alloys have been shown in experimental systems to produce non-neoplastic toxicity in different organs including the respiratory tract, the thyroid gland, erythropoietic tissue, myocardium and reproductive organs (Lison, 1996; National Toxicology Program, 1998; Barceloux, 1999). This section focuses on effects that may contribute to the evaluation of the carcinogenicity of inhaled hard-metal dusts and their components and is therefore limited mainly to studies examining effects on the respiratory tract.

(*a*) *Cobalt metal, hard metals and other alloys*

(i) *Inflammation and fibrosis: in-vivo studies*

A series of early experimental studies, initiated in the 1950s, explored the potential mechanisms of the respiratory diseases observed in workers in plants producing hard metal in Germany, the United Kingdom and the USA (see IARC, 1991). These studies

were essentially designed to compare the effects of cobalt metal or oxide, tungsten, tungsten carbide and hard-metal mixtures.

Rats

Harding (1950) was probably the first to describe severe and fatal pulmonary oedema and haemorrhage in piebald rats administered cobalt-metal powder by intratracheal instillation (500 μg/rat), and suggested that this acute pulmonary toxicity might be related to the high solubility of cobalt metal in protein-containing fluids, presumably through some attachment of cobalt metal to protein.

Kaplun and Mezencewa (1960) found that the lung toxicity induced in rats by a single intratracheal instillation of cobalt-metal particles (5 or 10 mg/animal) was exacerbated by the simultaneous addition of tungsten or titanium (10 mg of a mixture containing 8–15% cobalt). Examination of the lungs after 4, 6 and 8 months revealed that pathological changes induced by the mixtures were identical to those produced by cobalt alone but more marked. The authors described a 'thickening' of the lung parenchyma with accumulation of lymphocytes, histiocytes and fibroblasts, hyperplasia of the walls of airways and blood vessels, and the presence of adenomas occurring several months after a single dose. The enhanced toxicity of the tungsten carbide–cobalt mixture was explained by the higher solubility of cobalt in the presence of tungsten (4–5-fold increase in 0.3% HCl during 24 h) (Kaplun & Mezencewa, 1960).

Kitamura *et al.* (1980) examined the pulmonary response of male Sprague-Dawley rats to a single administration of cemented tungsten carbide powder obtained after grinding pre-sintered alloy with diamond wheels. The powder was administered intratracheally at a dose of 23 mg/100 g bw. About 20% of the animals died during the first 3 days after exposure; histological examination of the lungs revealed marked haemorrhagic oedema with intense alveolar congestion. Among survivors, a transient reduction in body weight gain was also observed during the first week post-exposure. Six months after exposure, all sacrificed animals showed pulmonary lesions of patchy fibrosis in the vicinity of deposited dust (peribronchiolar and perivascular regions), occasionally associated with traction emphysema. There was no definitive inflammatory reaction nor interstitial pneumonitis (alveolitis). The lesions were suggested to result from condensation of collapsed alveoli without noticeable dense collagenization. In rats sacrificed at 12 months, the lesions had apparently regressed and two-thirds of the animals had neither fibrosis nor dust retention; the remaining animals showed changes similar to those observed at 6 months. The toxic effect on the lung was attributed, without experimental evidence, to the cytotoxic action of cobalt released from the particles. Neither cobalt metal nor tungsten carbide alone were tested.

Tozawa *et al.* (1981) examined the lung response to pre-sintered cemented carbides (WC:Co, 98:2 or WC:Co:TiC:TaC, 64:16:6:14) in male Sprague-Dawley rats, 6 and 12 months after a single intratracheal administration. They observed marked fibrotic foci after 6 months that were to some extent reversed 6 months later. They also noted that cobalt was eliminated more rapidly than tungsten from the lung. Neither cobalt metal nor tungsten carbide alone were tested.

Lasfargues *et al.* (1992) carried out studies in female Sprague-Dawley rats to compare the acute toxicity of hard-metal particles (WC–Co mixture containing 6% of cobalt-metal particles; d_{50}, 2 μm) with tungsten carbide particles (WC; cobalt content, 0.002%) and with an equivalent dose of cobalt-metal particles alone. After intratracheal instillation of a high dose of cobalt-metal particles (1 mg/100 g bw; median particle size d_{50}, 4 μm), a significantly increased lung weight was noted at 48 h. The lung weights of the animals exposed to WC (15.67 mg/100 g bw) were no different from those of control rats, but significant increases were noted in animals exposed to the hard metal (16.67 mg/100 g bw). These increases were much more substantial in the WC–Co group than in those animals instilled with an equivalent dose of cobalt particles alone. Increased mortality was observed in the group of animals exposed to WC–Co but not in those instilled with cobalt metal or WC alone. A second series of experiments with non-lethal doses (cobalt metal, 0.06 mg/100 g bw; tungsten carbide particles, 1 mg/100 g bw; hard-metal mixture, 1 mg/100 mg bw) was performed in order to analyse the cellular fraction of BALF and lung histology 24 h after dosing. While histological lung sections from rats instilled with cobalt alone or tungsten carbide particles were almost normal, an intense alveolitis was observed in rats exposed to the hard-metal mixture. In rats exposed to cobalt metal alone, no significant biochemical or cellular modifications in BALF were observed. Analysis of the cellular fraction of BALF from animals exposed to hard-metal particles showed a marked increase in the total cell number, similar to that induced by the same dose of crystalline silica; the increase in the neutrophil fraction was even more pronounced than that in the silica-treated group. Similarly, biochemical analyses of the cell-free fraction of BALF showed an increase in lactate dehydrogenase (LDH) activity, total protein and albumin concentration in the group instilled with hard metal, while exposure to the individual components of the mixture, i.e. Co or WC, did not produce any significant modification of these parameters (Lasfargues *et al.*, 1992). No change in the ex-vivo production of the inflammatory mediators interleukin-1 (IL-1) and TNF-α, a growth factor fibronectin or a proteinase inhibitor cystatin-c by lung phagocytes was found 24 h after administration of cobalt metal (0.06 mg/100 g bw), WC (1 mg/100 g bw) and WC–Co (1 mg/100 g bw) (Huaux *et al.*, 1995).

Lasfargues *et al.* (1995) also examined the delayed responses after single intratracheal administrations of tungsten carbide or hard-metal particles (WC or WC–Co, 1, 5 or 10 mg/100 g bw) or cobalt-metal particles (0.06, 0.3 or 0.6 mg/100 g bw) alone. The lung response to the hard-metal mixture was characterized by an immediate toxic response (increased cellularity and LDH, *N*-acetylglucosaminidase, total protein and albumin concentrations) in BALF followed by a subacute response after 28 days. The effects of cobalt or tungsten carbide alone were very modest, occurring at the highest doses only. Four months after instillation, fibrosis could not be identified histologically in the lungs of the animals treated with the hard-metal powder. This reversibility of the lesions was considered reminiscent of the natural history of hard-metal disease in humans. After repeated intratracheal administrations (once a month for 4 months) of the different particles (1 mg/100 g bw WC or WC–Co, or 0.06, 0.3 or 0.6 mg/100 g bw cobalt), no effect on the lung parenchymal architecture was observed in the groups treated with tungsten carbide or cobalt alone. In contrast,

clear fibrotic lesions were observed in the group instilled with hard metal. No giant multinucleated cells were observed in BALF nor lung tissue of animals treated with WC–Co.

Kyono *et al.* (1992) examined the effect of ultrafine cobalt-metal particles (mean diameter, 20 nm) on the lungs of Sprague-Dawley-Jcl rats exposed by inhalation (2 mg/m^3) for 5 h per day for 4 days. The rats were killed at 2 h, or at 3, 8 or 28 days after the end of exposure. Focal hypertrophy and proliferation of the lower airway epithelium, damaged macrophages and type I pneumocytes as well as proliferation of type II cells, fibroblasts and myofibroblasts were observed early after exposure. Morphological transformation of damaged type I cells to the 'juvenile' form (large nucleolus, abundant smooth endoplasmic reticulum, prominent Golgi apparatus and cytoplasm) was also reported, and interpreted as a sign of active biosynthesis and a capability of self-repair of this cell type. Cobalt was shown to be removed from the lung in two phases with estimated half-lives of 53 and 156 h, respectively. The morphological lesions caused by ultrafine cobalt under the presented conditions were reversible after 1 month: severe fibrosis was not detected in the lungs examined at 28 days. In a companion study, a single intratracheal instillation of ultrafine cobalt metal (0.5 or 2 mg) into rats caused alveolar septal fibrosis detectable 15 months after treatment. Therefore, the authors noted that the possibility that fibrosis can develop after prolonged exposure to ultrafine cobalt metal must be considered.

Adamis *et al.* (1997) examined the lung response in male Sprague-Dawley rats exposed to respirable dust samples collected at various stages of hard-metal production in a plant in Hungary. Samples included finished powder for pressing (8% cobalt content), heat-treated, pre-sintered material (8% cobalt) and wet grinding of sintered hard metal (3% cobalt). The animals were administered 1 and/or 3 mg of dust suspended in saline and were killed after 1, 4, 7 or 30 days. Analyses of BALF (LDH, acid phosphatase protein and phospholipids) indicated the occurrence of an inflammatory reaction, a damage of the cell membrane and an increase of capillary permeability which varied with the type of powder used, with the pre-sintered sample showing the greatest toxicity. Histological studies showed that the pathological changes induced by the three powders were essentially the same, consisting of oedema, neutrophil and lymphocyte infiltration, together with an accumulation of argyrophilic fibres in the interalveolar septa and in the lumina of alveoli and bronchioli.

Zhang *et al.* (1998) compared ultrafine cobalt-metal particles (mean diameter, 20 nm; 47.9 m^2/g surface area) with ultrafine nickel and titanium dioxide powders for their capacity to produce inflammation after a single intratracheal instillation into male Wistar rats (1 mg/animal). All indices measured in BALF indicated that ultrafine nickel was the most toxic material. In the group of animals treated with cobalt particles, the lung:body weight ratio was significantly increased at days 1, 3, 7 and 15 after exposure and returned to normal after 30 days; LDH activity, total protein, lipid peroxide concentrations and inflammatory cells in BALF were significantly increased for up to 30 days.

Guinea-pigs

A single intratracheal instillation of cobalt metal (10–50 mg) into guinea-pigs [strain not specified] was shown to result in the development of acute pneumonia with diffuse

eosinophilic infiltration and bronchiolitis obliterans. The subchronic response assessed 8–12 months after the dose was characterized by the presence of multinucleated cells and a lack of cellular reaction within the alveolar walls. It was concluded that cobalt metal is not fibrogenic and does not provoke a chronic lesion in the regional lymph nodes (Delahant, 1955; Schepers, 1955a).

In the comparative studies (Schepers, 1955b), instillation of cobalt metal mixed with tungsten carbide (150 mg in a 9:91 ratio, i.e. a dose of 13.5 mg cobalt metal) into guinea-pigs [strain not specified] induced a transient inflammatory reaction with residual papillary hypertrophy of bronchial mucosa and peribronchial and periarterial fibrosis in the vicinity of retained particles. In inhalation experiments, a similar mixture (ratio 1:3) caused severe inflammation with focal pneumonia and bronchial hyperplasia and metaplasia, but fibrosing alveolitis was not observed after treatment with the cobalt metal–tungsten carbide mixture. Multinucleated giant cells were also found in animals treated with a combination of tungsten carbide and carbon (without cobalt); tungsten carbide alone was not tested (Schepers, 1955c).

Rabbits

Exposure of rabbits by inhalation to cobalt metal (0.2 and 1.3 mg/m^3) for 4 weeks did not produce any inflammatory reaction; the particles were not taken up by macrophages and the phagocytic capacity of these cells was not impaired (Johansson *et al.*, 1980).

Mini-pigs

Kerfoot *et al.* (1975) exposed mini-pigs for 3 months (6 h per day, 5 days per week) to aerosols of cobalt metal at concentrations of 0.1 and 1.0 mg/m^3. The animals were first submitted to a sensitization period of 5 days' exposure to cobalt followed by a 10-day removal from exposure before the 3 months of exposure. Post-exposure lung function studies demonstrated a dose-dependent and reversible reduction in lung compliance but no radiographic or histological signs of fibrosis, except some increased collagen deposition which could only be detected electron-microscopically. The authors interpreted these changes as demonstrating functional impairment. Functional alterations were no longer detectable 2 months after the end of cobalt exposure. [The Working Group noted that the collagen increase was not assessed quantitatively.]

(ii) *Cytotoxicity: in-vitro studies*

Mouse macrophage cells

Cytotoxic effects on mouse peritoneal macrophages of a range of metallic particles of orthopaedic interest have been examined. High doses of cobalt metal and cobalt–chromium alloy (0.5 mg metal/2 mL/3 × 10^6 cells) caused membrane damage as indicated by the increased release of cytoplasmic LDH. Decreased glucose-6-phosphate dehydrogenase (G6PD) activity was also observed after 10 h incubation with the same dose of metal (Rae, 1975).

In mouse peritoneal and alveolar macrophages, Lison and Lauwerys (1990) showed that the cytotoxicity of cobalt-metal particles, assessed by LDH release and morphological examination, was significantly increased in the presence of tungsten carbide particles either as industrial hard-metal powders or a WC–Co mixture reconstituted in the laboratory (6% cobalt in weight). Both particles (Co and WC) needed to be present simultaneously in order to exert their increased cytotoxic action. The interaction between tungsten carbide and cobalt particles was associated with an increased solubilization of cobalt in the culture medium. However, in the test system used, the toxicity of hard-metal particles could not be ascribed to solubilized cobalt ions, because the effects could not be reproduced with cobalt chloride or with cobalt ions solubilized from the WC–Co mixture (Lison & Lauwerys, 1992). In further studies, the cytotoxic potential of cobalt-metal particles, based on the measurement of glucose uptake, G6PD activity and superoxide anion production, was assessed *in vitro* in mouse peritoneal and alveolar macrophages incubated in a culture medium supplemented with 0.1% lactalbumin hydrolysate. Glucose uptake and superoxide anion production were significantly more depressed by a WC–Co mixture than by cobalt alone, while G6PD activity was decreased by both WC–Co and cobalt-metal particles alone (Lison & Lauwerys, 1991). Cobalt-metal particles (d_{50}, 4 μm and 12 μm; 10–100 μg/10^6 cells) affected cell integrity only marginally (Lison & Lauwerys, 1991).

Using the LDH release assay in mouse peritoneal macrophages, a similar toxic interaction between cobalt-metal particles and other metallic carbides (titanium, niobium and chromium carbides) but not latex beads (Lison & Lauwerys, 1992), crystalline silica, iron or diamond particles, was found. It was noted that the interaction between the carbides and the cobalt particles was dependent to some extent on the specific surface area of the particles suggesting the involvement of a surface chemistry (physicochemical) phenomenon (Lison & Lauwerys, 1995).

Lison *et al.* (1995) found that butylated hydroxytoluene protected macrophage cultures from the toxicity of a WC–Co (94:6) mixture, suggesting the involvement of lipid peroxidation in the cytotoxic activity of these particles. Lipid peroxidation was also demonstrated by the formation of thiobarbituric acid-reactive substances when arachidonic acid was incubated with WC–Co particles. Lison and Lauwerys (1993) had shown earlier that other enzymes that detoxify activated oxygen species such as catalase and superoxide dismutase (SOD), and scavengers such as sodium azide, benzoate, mannitol, taurine or methionine, did not protect against the cytotoxicity of WC–Co particles.

Rat fibroblasts, alveolar macrophages and type II pneumocyte cells

Thomas and Evans (1986) observed no effect of a cobalt–chromium–molybdenum alloy (0.5–10 mg/mL) on the proliferation of rat fibroblasts in culture nor on production of collagen.

Roesems *et al.* (1997), using the same experimental model as Lison and Lauwerys (1990; see above) but incubating the preparations in the absence of lactalbumin hydrolysate, which was replaced by foetal calf serum, showed that rat alveolar type II pneumocytes were less sensitive than alveolar macrophages to cobalt-metal particles *in vitro*, and

that human type II pneumocytes were even less sensitive than rat type II pneumocytes. In contrast, using the dimethylthiazol diphenyl tetrazolium (MTT) assay, rat type II pneumocytes were found to be more sensitive than alveolar macrophages (25 μg/600 000 cells) and the toxicity of cobalt-metal particles could be reproduced by cobalt ions (Roesems *et al.*, 2000).

In the experimental system used by Roesems *et al.* (2000), an increased cytotoxicity of cobalt-metal particles associated with WC was confirmed in rat alveolar macrophage cell cultures, but not in type II pneumocytes. In contrast to the results presented by Lison and Lauwerys (1992), Roesems *et al.* (2000) found that cobalt ions played a role in the cytotoxic effect of cobalt-metal particles whether associated or not with WC. This difference was probably due to the presence of lactalbumin hydrolysate which was found to quench cobalt ions and may have masked their cytotoxicity in the experiments by Lison and Lauwerys (1992) and Lison (2000). *In vivo*, however, the bioavailability of cobalt ions is relatively limited because these cations precipitate in the presence of physiological concentrations of phosphates ($Co_3(PO_4)_2$; K_{sp}: 2.5×10^{-35} at 25 °C) (Lison *et al.*, 1995; 2001) and bind to proteins such as albumin (Merritt *et al.*, 1984).

In rat type II pneumocytes, cobalt-metal particles (15–1200 μg/3 × 10^6 cells) were found to stimulate the hexose monophosphate shunt in a dose- and time-dependent manner, indicating that these particles caused oxidative stress (Hoet *et al.*, 2002).

(iii) *Biochemical effects*

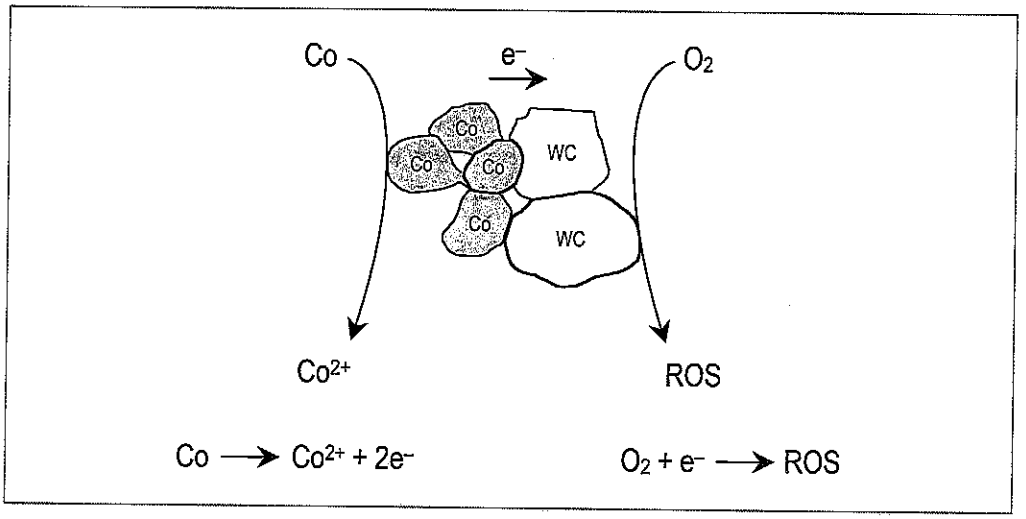
As a transition element, cobalt shares a number of chemical properties with iron and thus it has been suggested that it may catalyse the decomposition of hydrogen peroxide by a Fenton-like mechanism. While several studies have indeed indicated that reactive oxygen species (ROS) are formed in the presence of a mixture of cobalt(II) ions and hydrogen peroxide (Moorhouse *et al.*, 1985), the exact nature of the radicals formed is still a matter of speculation. These free radicals have been proposed to account for several toxic properties of cobalt compounds, including their genotoxic activity.

Lison and Lauwerys (1993) using a deoxyribose degradation assay reported a significant formation of hydroxyl radicals *in vitro* when cobalt-metal particles (d_{50}, 4 μm; 6 μg/mL) were incubated with hydrogen peroxide. However, this effect was less than that seen with an equivalent concentration of cobalt(II) ions. The activity of cobalt-metal particles was increased about threefold when associated with tungsten carbide particles (d_{50}, 2 μm). It was also noted that the latter behaved as a strong oxidizing compound, but the exact role of this activity in the interaction with cobalt metal or cobalt(II) ions could not be elucidated.

Using electron spin resonance (ESR) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) spin trapping in a cell-free system, Lison *et al.* (1995) reported that cobalt-metal particles (d_{50}, 4 μm; 1 mg/mL phosphate buffer) produced small amounts of activated oxygen species, presumed to be hydroxyl radicals. This activity was observed in the absence of hydrogen peroxide and could not be reproduced with cobalt(II) ions, indicating that a Fenton-like mechanism was not involved. The production of activated oxygen species by

cobalt-metal particles was markedly increased in the presence of tungsten carbide particles (Co:WC, 6:94). It was proposed that this reaction could be the consequence of a solid-solid interaction between particles whereby molecular oxygen is reduced at the surface of WC particles by electrons migrating from cobalt-metal particles, which are consequently oxidized and solubilized. The resulting Co(II) did not drive the production of ROS (see Figure 2). Further investigations of the surface interaction between cobalt metal and tungsten carbide particles (Zanetti & Fubini, 1997) indicated that the association of the two solids behaves like a new chemical entity, with physico-chemical properties different from those of the individual components, and which provides a lasting source of ROS as long as metallic cobalt is present. Radical generation originates from reactive oxygen formed at the carbide surface. When compared to other metals (iron, nickel), cobalt metal was the most active in the above reaction (Fenoglio *et al.*, 2000). In the presence of hydrogen peroxide, the WC–Co mixture exhibits a peroxidase-like activity (Fenoglio *et al.*, 2000; Prandi, 2002).

Figure 2. Mechanism proposed for release of reactive oxygen species (ROS) from buffered aqueous suspensions of cobalt/tungsten carbide (Co/WC) mixtures (hard metals)



Adapted from Zanetti & Fubini (1997)
Cobalt is progressively oxidized and solubilized; oxygen is activated at the carbide surface.
e^-, electron

Leonard *et al.* (1998), using ESR, confirmed that cobalt-metal particles in aqueous suspension reduced molecular oxygen. The authors proposed that the species generated is likely to be a cobalt(I)-bound superoxide anion (Co(I)-OO•) which exhibits strong oxidizing properties. This product was further shown in the presence of SOD, to generate hydrogen peroxide which reacts with Co(I) to produce a hydroxyl radical and Co(II) via a Co(I)-

mediated Fenton-like reaction. In the presence of proper chelators, such as glutathione (GSH), Gly-Gly-His and anserine, the cobalt(II) ions formed by the molecular oxygen oxidation of cobalt produce hydroxyl radicals and Co(III) through a Co(II)-mediated Fenton-like mechanism.

Keane *et al.* (2002) confirmed the generation of hydroxyl radicals by hard-metal materials in aqueous suspension by examining the properties of detonation coating, a hard metal-related material made of a WC and cobalt mixture (6.7 and 5.4% of cobalt in pre- and post-detonation powders, respectively). The post-detonation powder was a much stronger generator of hydroxyl radicals than the pre-detonation material.

(*b*) *Other relevant cobalt compounds*

(i) *Inflammation and fibrosis: in-vivo studies*

In a dose-finding study for a carcinogenicity assay (Bucher *et al.*, 1990), male and female Fischer 344/N rats and B6C3F$_1$ mice were exposed to cobalt sulfate heptahydrate aerosols of 0, 0.3, 1.0, 3.0, 10 or 30 mg/m^3 for 6 h per day on 5 days per week for 13 weeks. The main histopathological effects in both species were limited to the respiratory tract. Lesions included degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, inflammation in the nose, and fibrosis, histiocytic infiltrates, bronchiolar epithelial regeneration and epithelial hyperplasia in the alveoli of the lungs. In rats, inflammation, necrosis, squamous metaplasia, ulcers and inflammatory polyps of the larynx were observed; mice developed metaplasia of the trachea. The most sensitive tissue in rats was the larynx: squamous metaplasia was observed with the lowest exposure concentration (0.3 mg/m^3). Degeneration of the olfactory epithelium was noted at the two highest doses tested (10 and 30 mg/m^3). A no-observed-adverse-effect level was not reached in these studies.

In the subsequent carcinogenicity study (0, 0.3, 1.0 or 3.0 mg/m^3 cobalt sulfate heptahydrate, 6 h per day, 5 days per week for 104 weeks) conducted in the same rodent strains (National Toxicology Program, 1998; Bucher *et al.*, 1999) (see also Section 3.1.1), similar non-neoplastic effects were noted. Degeneration of olfactory epithelium was more pronounced than in the 13-week dose-finding study and was observed at the lowest dose tested (0.3 mg/m^3). In rats, proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation and interstitial fibrosis were observed at all dose levels. The non-neoplastic lesions were less severe in mice and mainly consisted of cytoplasmic vacuolization of the bronchi. Diffuse and focal histiocytic cell infiltrations were also observed in lungs of mice with neoplasms and were therefore considered to be a consequence of the neoplasms rather than a primary effect of cobalt sulfate.

Wehner *et al.* (1977) examined the influence of lifetime inhalation of cobalt oxide (10 µg/L, 7 h/day, 5 days/week) alone or in combination with cigarette smoke in male Syrian golden hamsters with the aim of detecting a carcinogenic effect. No tumorigenic action of cobalt oxide was observed and there was no additive effect of exposure to smoke.

Some pulmonary changes consisting of focal interstitial fibrosis, granulomas, hyperplasia of alveolar cells and emphysema were observed in animals exposed to cobalt oxide alone.

Lewis *et al.* (1991) showed that the intratracheal instillation of cobalt chloride (1–1000 μg/kg) into Syrian golden hamster lungs induced biochemical changes compatible with the development of oxidative stress (i.e. decreased concentrations of reduced glutathione, increased concentrations of oxidized glutathione, and stimulation of the pentose phosphate pathway). Similar changes were also observed *in vitro* after incubation of lung slices with cobalt chloride (0.1–10 mM), and preceded the detection of cellular toxicity indicating their possible early involvement in the pulmonary toxicity of cobalt (II) ions. It was later shown in the same in-vitro model that simultaneous treatment with hydrogen peroxide or 1,3-bis(2-chloroethyl)-1-nitrosourea, a glutathione reductase inhibitor, potentiated the oxidative stress induced by cobalt chloride; however, this effect was not associated with an enhancement of cell dysfunction observed with cobalt chloride alone or cobalt chloride and hydrogen peroxide together. Furthermore, on the basis of comparative analysis of the results with the known oxidant tert-butyl hydroperoxide, glutathione oxidation did not appear to be the cause of the cellular dysfunction caused by cobalt chloride (Lewis *et al.*, 1992).

In a series of studies on the effects of various cobalt compounds on rabbit lung morphology (Johansson *et al.*, 1983, 1986), exposure to 0.4, 0.5 and 2.0 mg/m^3 soluble cobalt chloride for 1 and 4 months increased the number of alveolar macrophages and their oxidative metabolic activity. Exposure to 0.4 and 2 mg/m^3 cobalt chloride for 14–16 weeks (6 h/day, 5 days/week) induced a combination of lesions characterized by nodular aggregation of type II pneumocytes, abnormal accumulation of enlarged, vacuolated alveolar macrophages and interstitial inflammation (Johansson *et al.*, 1987). The effect of cobalt chloride (0.5 mg/m^3 for 4 months) on rabbit lung, i.e. formation of noduli of type II cells, was potentiated by simultaneous exposure to nickel chloride administered at the same dose (Johansson *et al.*, 1991). Camner *et al.* (1993) reported that the inflammatory reaction (as indicated by the presence of neutrophils and eosinophils in BALF) induced by the inhalation of cobalt chloride (2.4 mg/m^3, 6 h/day for 2 weeks) was more pronounced in guinea-pigs that had been pre-sensitized to cobalt by repeated application of cobalt chloride.

(ii) *Cytotoxicity: in-vitro studies*

At relatively high doses (0.1–1 mM), cobalt(II) ions have been shown to inhibit exocytosis and respiratory burst in rabbit neutrophils through an interaction with a calcium-dependent intracellular mechanism (Elferink & Deierkauf, 1989).

In U-937 cells and human alveolar macrophages, cobalt ions (0.5–1 mM as cobalt chloride) induced apoptosis and accumulation of ubiquitinated proteins. It was suggested that cobalt-induced apoptosis contributed to cobalt-induced lung injury (Araya *et al.*, 2002). In neuronal PC12 cells cobalt chloride triggered apoptosis in a dose- and time-dependent manner, presumably via the production of ROS and the increase of the DNA-binding activity of transcriptor factor AP-1 (Zou *et al.*, 2001). A subsequent study showed

that caspase-3 and p38 mitogen-activated protein kinase-mediated apoptosis was induced by cobalt chloride in PC12 cells (Zou *et al.*, 2002).

Microtubule disorganization has been reported in 3T3 cells exposed to high concentrations of cobalt sulfate (100 µM) for 16 h (Chou, 1989).

Soluble cobalt compounds (40 µM [5 µg/mL] as cobalt chloride), but not particulate materials, have been reported to induce cytotoxicity and neoplastic transformation in the C3H10T½ assay (Doran *et al.*, 1998) (see Section 4.4).

(iii) *Biochemical effects*

Using two assays to detect hydroxyl radicals (HO•), based either on the degradation of deoxyribose or the hydroxylation of phenol or salicylate, Moorhouse *et al.* (1985) found that, in an acellular system at physiological pH, cobalt(II) ions promoted the formation of hydroxyl-like radicals in the presence of hydrogen peroxide (H_2O_2, 1.44 mM); the formation of the radicals was decreased by catalase, but not by SOD or ascorbic acid. Ethylenediaminetetraacetic acid (EDTA) in excess of Co(II) accelerated the formation of ROS, and hydroxyl radical scavengers such as mannitol, sodium formate, ethanol or urea, blocked deoxyribose degradation by the cobalt(II)–H_2O_2 mixture. Lison and Lauwerys (1993) reported similar findings, i.e. a significant degradation of deoxyribose in the presence of cobalt(II) (0.1 mM) mixed with hydrogen peroxide (1.44 mM), suggesting the formation of hydroxyl radicals.

Using an ESR spin-trapping technique (with DMPO), Kadiiska *et al.* (1989) found that cobalt(II) ions, unlike iron(II) ions, did not react with hydrogen peroxide by the classic Fenton reaction at physiological pH, either in a chemical system or in rat liver microsomes. They suggested that superoxide anions, not hydroxy radicals, were primarily formed. In a subsequent study using the same technique, Hanna *et al.* (1992) used several ligands to complex cobalt(II) ions and further documented the formation of superoxide anions, but not hydroxyl radicals, in the presence of hydrogen peroxide.

Using ESR, Wang *et al.* (1993) detected the ascorbic acid radical *in vivo* in circulating blood after intravenous administration of ascorbic acid (100 mM) and cobalt(II) at two separate sites into male Sprague-Dawley rats. Similar but less intense signals were also observed with nickel(II) and iron(II) ions. The formation of the ascorbic acid radical was interpreted as the in-vivo formation of free radicals in animals overloaded with cobalt(II) ions; the mechanism of this radical formation was, however, not addressed. The authors suggested that their findings might explain the mechanism of the toxicity observed in workers exposed to cobalt-containing materials.

The in-vitro generation of ROS by cobalt(II) from hydrogen peroxide and related DNA damage have also been examined by Mao *et al.* (1996). The formation of hydroxyl radicals and/or singlet oxygen ($^1O_2$) showed that the oxidation potential of cobalt(II) could be modulated by several chelators such as anserine or 1,10-phenanthroline. Shi *et al.* (1993) examined the modulation of ROS production from cobalt(II) ions and hydroperoxides and showed that several chelating agents, including endogenous compounds such as reduced GSH, facilitated the production of these species.

Sarkar (1995) hypothesized that oligopeptides or proteins represent other ligands that can modulate the redox potential of cobalt(II) ions. The presence of such proteins (histones) in the nucleus might allow the production of ROS in close proximity to biologically-relevant targets such as DNA. It has also been suggested that the ability of cobalt(II) to substitute for zinc(II) in the DNA-binding domain of nuclear (transcription factor) proteins might allow the in-situ formation of free radicals that may damage genetic regulatory/response elements and may explain the mutagenic potential of these metals.

(iv) *Other effects*

Cobalt also interferes with cellular mechanisms that control the degradation of regulatory proteins such as p53, which is involved in the control of the cell cycle, genome maintenance and apoptosis. An *et al.* (1998) reported that, in mammalian cells, cobalt chloride (100 μM) activates hypoxia-inducible factor-1α which in turn induces accumulation of p53 through direct association of the two proteins. Cobalt sulfate (50 μg/mL [178 μM]) has been shown to induce p53 proteins in mouse cells treated *in vitro* (Duerksen-Hughes *et al.*, 1999). Inhibition of proteasome activity by cobalt (1 mM), subsequent accumulation of ubiquitinated proteins and increased apoptosis have been reported in human alveolar macrophages and U-937 cells (Araya *et al.*, 2002) (See Section 4.4.2). Whether these biochemical mechanisms are involved in the carcinogenic responses observed with some cobalt compounds remains, however, to be examined.

4.3 Reproductive and developmental effects

Only a few studies have been conducted with soluble cobalt compounds to explore their potential effects on development.

Wide (1984) reported that a single intravenous injection of cobalt chloride hexahydrate into pregnant NMRI mice (5 mM per animal in the tail vein; [120 μg/animal]) on day 8 of gestation significantly affected fetal development (71% of skeletal malformations versus 30% in controls); in animals injected at day 3 of gestation, no interference with implantation was noted. In the same experiment but replacing cobalt chloride by tungstate (25 mM of W per animal; [460 μg/animal]) a significant increase in the number of resorptions was observed (19% versus 7% in controls), but no skeletal malformations.

In a study undertaken by Pedigo and colleagues (1988), following 13 weeks of chronic exposure to 100 to 400 ppm [100–400 μg/mL] cobalt chloride in drinking water, male CD-1 mice showed marked dose-related decreases in fertility, testicular weight, sperm concentration and motility, and increases in circulating levels of testosterone. Pedigo and Vernon (1993) reported that cobalt chloride (400 ppm in drinking-water for 10 weeks) increased pre-implantation losses per pregnant female in the dominant lethal assay by compromising the fertility of treated male mice.

Paksy *et al.* (1999) found that in-vitro incubation of postblastocyst mouse embryos with cobalt(II) ions (as cobalt sulfate) adversely affected the development stages at a concentration of 100 μM and decreased the trophoblast area (at a concentration of 10 μM).

In pregnant Wistar rats, oral administration of cobalt(II) ions as cobalt chloride (12, 24 or 48 mg/kg bw per day from day 14 of gestation through to day 21 of lactation) significantly affected the late period of gestation as well as postnatal survival and development of the pups. Signs of maternal toxicity were apparently also noted but the details are not reported (Domingo *et al.*, 1985).

A study conducted in pregnant Sprague-Dawley rats (Paternain *et al.*, 1988) concluded that the administration of cobalt chloride (up to a dose of 100 mg/kg by gavage, from day 6–15 of gestation) was not embryotoxic nor teratogenic, despite signs of maternal toxicity.

Sprague-Dawley rats maintained on diets (15 g per day) containing 265 ppm [31.8 mg/kg bw per day] cobalt for up to 98 days showed degenerative changes in the testis from day 70 to the end of the treatment; given that cobalt was not detected in testis, these changes were considered secondary to hypoxia due to blockage of veins and arteries by red blood cells and changes in permeability of the vasculature and seminiferous tubules (Mollenhaur *et al.*, 1985). Decreased sperm motility and/or increased numbers of abnormal sperm were noted in mice, but not in rats, exposed to 3 mg/m^3 or higher concentrations (30 mg/m^3) in 13-week inhalation studies with cobalt sulfate (National Toxicology Program, 1991).

The fetal and postnatal developmental effects of cobalt sulfate have been compared in C57BL mice, Sprague-Dawley rats and/or New Zealand rabbits (Szakmáry *et al.*, 2001). Several developmental alterations (elevated frequency of fetuses with body weight or skeletal retardation, embryolethality, increased anomalies in several organs) were observed in pregnant mice and rats treated with cobalt sulfate by gavage on days 1–20 of gestation (25, 50 or 100 mg/kg bw per day, respectively). In rabbits, cobalt sulfate at 20 mg/kg bw was embryotoxic with inhibition of skeletal development. No teratogenic effects were noted in rabbits treated with up to 200 mg/kg per day during days 6–20 of gestation. Postnatal developmental parameters were transiently altered in the pups of rats treated daily with 25 mg/kg cobalt sulfate. [The Working Group noted that the doses used in these studies were relatively high and produced maternal toxicity. The interpretation of these data should, therefore, be considered with caution].

4.4 Genetic and related effects

4.4.1 *Humans*

(*a*) *Sister chromatid exchange*

Five studies have been conducted to date on the possible cytogenetic effects induced by cobalt compounds in lymphocytes (or leukocytes) of individuals exposed to metals.

Results of sister chromatid exchange have been obtained in two studies in which exposure was to a mixture of metals. Occupational exposure to metals was studied by Gennart *et al.* (1993) who determined sister chromatid exchange in 26 male workers (aged 23–59 years) exposed to cobalt, chromium, nickel and iron dust in a factory produ-

cing metal powder and in 25 controls (aged 24–59 years), who were clerical workers, matched for age, smoking habits and alcohol consumption. The metal particle sizes ranged from 2 to 100 μm. Slight exposure to nickel or chromium oxides could not be excluded, since, at one stage of the production process, the metals are melted in an oven. The workers had been employed for at least 2 years (range, 2–20 years). The atmospheric concentrations of cobalt were measured at two different work areas in 1986 and in 1989, at the time of the cytogenetic survey. An improvement in the local exhaust ventilation system took place between the two sampling times. At the work area where the ovens were located, the (geometric) mean cobalt concentration in the air (based on 4–8 values) was 92 $\mu g/m^3$ in 1986 and 40 $\mu g/m^3$ in 1989. At the second work area, the individual values ranged from 110 to 164 $\mu g/m^3$ in 1986 and from 10 to 12 $\mu g/m^3$ in 1989. The differences in the concentrations of cobalt in the urine in exposed persons (cobalt geometric mean, 23.6 μg/g creatinine; range, 6.4–173.1) and controls (cobalt geometric mean, 1.1 μg/g creatinine; range, 0.2–3.2) were statistically significant. Analysis of variance revealed that both exposure status (exposed versus controls) and smoking habits (smokers and former smokers versus never smokers) had statistically-significant effects on the sister chromatid exchange or high-frequency cell (HFC) rank values. These effects may not be attributable to cobalt alone.

Stea *et al.* (2000) compared sister chromatid exchange in patients who had chrome–cobalt alloy prostheses and in those with other metal alloys. The study population consisted of 30 patients (11 men and 19 women; mean age, 63.8 years; range, 33–78) with joint (28 hip and two knee) prostheses and 17 control subjects (11 men and six women; mean age, 58.65 years; range, 40–71) matched for age, sex, and exposure to occupational and environmental risk factors such as chemicals, antineoplastic drugs and traffic smog. Ten subjects (mean age, 65.1 years; range, 51–76) had prostheses made of titanium–aluminium–vanadium alloys, 14 subjects (mean age, 61.9 years; range, 33–75) had prostheses made of chrome–cobalt alloys and five (mean age, 65 years; range, 57–78) had mixed prostheses. Of the prostheses, 13 were cemented (in some cases only one component was cemented) and 17 were cementless. The average duration of the implant was 7.5 years (range, 0.5–25) for the hip prostheses and 2.5 years for the two knee prostheses. The mean sister chromatid exchange rate in subjects with prostheses (5.2 ± 1.5) was not statistically different from that in subjects without prostheses (4.4 ± 1.3). Subjects with titanium–aluminium–vanadium alloy prostheses had a significantly higher sister chromatid exchange frequency (6.3 ± 2.3) than the controls (4.4 ± 1.3) whereas subjects with prostheses made of chrome–cobalt alloys or mixed prostheses had a higher, but not significantly, sister chromatid exchange frequency (4.7 ± 1.1 and 5.0 ± 2.1, respectively) than the controls. The number of sister chromatid exchanges was not affected by the presence of bone-cement used in prosthesis fixation nor by duration of the implant. There was no difference in the incidence of sister chromatid exchange between the two populations (those with prostheses and controls) considered globally and the considered risk factors, including smoking. The HFC values (> 9 exchanges per cell) were also recorded. Among the cases studied, three patients with implants (one with a prosthesis made of chrome–cobalt alloy and two with

mixed prostheses) showed markedly elevated percentages of HFCs (> 10%). It was concluded that the indication of possible cytogenetic damage in the patient populations should be considered with caution, since the sample population was small.

(*b*) *Micronuclei, DNA damage*

Burgaz *et al.* (2002) applied the micronucleus test to assess the effect of occupational exposure to metal alloys in both exfoliated nasal cells, and *in vitro* in lymphocytes, with the cytochalasin-B technology which allows discrimination between lymphocytes that have divided once during the in-vitro culture periods (binucleates) and those that have not (mononucleates). The groups studied consisted of 27 male dental laboratory technicians (mean age, 29.2 ± 10.8 years) exposed to metal alloys (35–65% cobalt, 20–30% chromium, 0–30% nickel) in dental laboratories during the production of skeletal prostheses, and 15 male controls (mean age, 28.4 ± 9.5 years) from the faculty of pharmacy. The differences in concentrations of cobalt in urine of technicians and controls were statistically significant (urinary cobalt, 0.12 ± 0.24 μg/g creatinine in controls and 24.8 ± 24.1 μg/g creatinine in technicians). The mean frequencies of micronucleated binucleates among peripheral lymphocytes were significantly higher (4.00 ± 2.98) in the dental technicians than in controls (1.40 ± 1.30). A statistically-significant difference was also found between the mean frequencies of micronuclei in nasal cells among the dental technicians (3.5 ± 1.80) and the controls (1.19 ± 0.53). The correlation between duration of exposure (13.1 ± 9.1 years) and frequencies of micronuclei was statistically significant in lymphocytes, but not in nasal cells of technicians. The results of multifactorial variance analysis revealed that occupational exposure was the only factor that significantly influenced the induction of micronuclei. In the exposed group, a significant correlation was found between urinary cobalt concentrations and frequencies of micronuclei in nasal cells, but not in lymphocytes.

The possible genotoxic effects of occupational exposure to cobalt alone or to hard-metal dust (WC–Co) was explored in a study using the in-vitro cytochalasin-B micronucleus test in lymphocytes as end-point for mutations (De Boeck *et al.*, 2000). Micronuclei were scored both as binucleates and as mononucleates to discriminate between micronuclei accumulated during chronic exposure *in vivo* (mononucleates) and additional micronuclei expressed during the culture period *in vitro* (binucleates). The authors aimed to assess genotoxic effects in workers from cobalt refineries and hard-metal plants who were exposed at the time of the study to the TLV/time-weighted average (TWA) of cobalt-containing dust. The study comprised three groups of male workers: 35 workers (mean age, 38.5 ± 7.7 years; range, 27.7–55.3) exposed to cobalt dust from three refineries, 29 workers (mean age, 40.7 ± 12.4 years; range, 20.7–63.6) exposed to hard-metal dust (WC–Co) from two production plants and 27 matched control subjects (mean age, 38.0 ± 8.8 years; range, 23.3–56.4) recruited from the respective plants. In these three groups, the (geometric) mean concentration of cobalt in urine was 21.5 μg/g creatinine (range, 5.0–82.5) in workers exposed to cobalt, 19.9 μg/g creatinine (range, 4.0–129.9) in workers exposed to hard-metal dust and 1.7 μg/g creatinine (range, 0.6–5.5) in controls. The study design integrated additional complementary biomarkers of DNA damage: 8-hydroxy-

deoxyguanosine (8-OHdG) in urine, DNA single-strand breaks and formamido-pyrimidine DNA glycosylase (FPG)-sensitive sites with the alkaline Comet assay in mononuclear leukocytes. No significant increase in genotoxic effects was detected in workers exposed to cobalt-containing dust compared with controls. No difference in any genotoxicity biomarker was found between workers exposed to cobalt and to hard-metal dusts. The only statistically-significant difference observed was a higher frequency of micronucleated binucleate cytokinesis-blocked lymphocytes in workers exposed to cobalt compared to workers exposed to hard-metal dusts, but not in comparison with their concurrent controls. The frequency of micronucleated mononucleates did not vary among the different worker groups. Multiple regression analysis indicated that workers who smoked and were exposed to hard-metal dusts had elevated 8-OHdG and micronucleated mononucleate values. The authors concluded that workers exposed solely to cobalt-containing dust at TLV/TWA (20 μg cobalt/g creatinine in urine, equivalent to TWA exposure to 20 μg/m^3) did not show increased genotoxic effects but that workers who smoked and were exposed to hard-metal dusts form a specific occupational group which needs closer medical surveillance.

Hengstler *et al.* (2003) concluded from a study of workers co-exposed to cadmium, cobalt, lead and other heavy metals, that such mixed exposure may have genotoxic effects. The authors determined DNA single-strand break induction by the alkaline elution method in cryopreserved mononuclear blood cells of 78 individuals co-exposed to cadmium (range of concentrations in air, 0.05–138 μg/m^3), cobalt (range, 0–10 μg/m^3) and lead (range, 0–125 μg/m^3) and of 22 subjects without occupational exposure to heavy metals (control group). Non-parametric correlation analysis showed significant correlations between DNA single-strand breaks and cobalt ($p < 0.001$; $r = 0.401$) and cadmium ($p = 0.001$; $r = 0.371$) concentrations in air, but not lead concentrations. They elaborated a model with a logistic regression analysis and concluded from it that more than multiplicative effects existed for co-exposure to cadmium, cobalt and lead. Some concerns about the study were addressed by Kirsch-Volders and Lison (2003) who concluded that it did not provide convincing evidence to support the alarming conclusion of Hengstler *et al.* (2003).

4.4.2 *Experimental systems* (see Table 15 for references)

(*a*) *Metallic cobalt*

The results of tests for genetic and related effects of metallic cobalt, cobalt-metal alloys and cobalt (II) and (III) salts, with references, are given in Table 15.

Cobalt metal is active not only as a solid particle but also as a soluble compound.

The genetic toxicology of cobalt compounds has been reviewed by Domingo (1989), Jensen and Tüchsen (1990), Léonard and Lauwerys (1990), Beyersmann and Hartwig (1992), Hartwig (1995), Lison *et al.* (2001), National Institute of Environmental Health Sciences (2002) and De Boeck *et al.* (2003a). A report of the European Congress on Cobalt and Hard Metal Disease, summarizing the state of the art was published by Sabbioni *et al.* (1994b). The interactions of cobalt compounds with DNA repair processes (Hartwig, 1998;

Table 15. Genetic and related effects of cobalt

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt				
DNA breaks, alkaline elution, purified DNA (3T3 mouse cells)	+[c]		1 μg/mL (d_{50} = 4 μm)	Anard *et al.* (1997)
DNA breaks, alkaline elution, purified DNA (3T3 mouse cells)	r[c,d]		1 μg/mL + Na formate (d_{50} = 4 μm)	Anard *et al.* (1997)
Cell transformation, C3H10T1/2 mouse fibroblast cells, *in vitro*	–[c]		500 μg/mL ($d_{50} \leq 5$ μm)	Doran *et al.* (1998)
Induction of FPG-sensitive sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	–[e]		6 μg/mL (d_{50} = 4 μm)	De Boeck *et al.* (1998)
DNA breaks, alkaline elution, human lymphocytes, *in vitro*	+[c]		3 μg/mL (d_{50} = 4 μm)	Anard *et al.* (1997)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	+[c]		4.5 μg/mL (d_{50} = 4 μm)	Anard *et al.* (1997)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	+[c]		0.6 μg/mL (d_{50} = 4 μm)	Van Goethem *et al.* (1997)
DNA single-strand breaks and alkali labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	+[c]		0.3 μg/mL (d_{50} = 4 μm)	De Boeck *et al.* (1998)
DNA single-strand breaks and alkali labile sites, human mononuclear leukocytes, *in vitro*	+		0.6 μg/mL (d_{50} = 4 μm)	De Boeck *et al.* (2003b)
DNA repair inhibition, alkaline Comet Assay, human mononuclear leukocytes, *in vitro*	+		5.5 μg/mL MMS, post-treatment 1.2 μg/mL Co (d_{50} = 4 μm)	De Boeck *et al.* (1998)
DNA repair inhibition, alkaline Comet Assay, human mononuclear leukocytes, *in vitro*	+		co-exposure 5.5 μg/mL MMS, 1.2 μg/mL Co (d_{50} = 4 μm)	De Boeck *et al.* (1998)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+[c]		0.6 μg/mL (d_{50} = 4 μm)	Van Goethem *et al.* (1997)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+		3 μg/mL (d_{50} = 4 μm)	De Boeck *et al.* (2003b)
Cell transformation (foci), human non-tumorigenic osteosarcoma osteoblast-like *in vitro*	–		3 μg/mL (d_{50} = 1–4 μm)	Miller *et al.* (2001)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt alloys				
Co-Cr alloy				
Cell transformation, C3H10T1/2 mouse fibroblast cells, *in vitro*	–[c]		500 µg/mL ($d_5 \leq 5$ µm)	Doran *et al.* (1998)
rW-Ni-Co alloy				
DNA single-strand breaks, alkaline elution, human non-tumorigenic osteosarcoma cells, *in vitro*	+		5 mg/mL (d_{50} = 1–5 µm)	Miller *et al.* (2002)
Sister chromatid exchange, human non-tumorigenic osteosarcoma cells, *in vitro*	+		5 mg/mL (d_{50} = 1–5 µm)	Miller *et al.* (2002)
Micronucleus formation, human non-tumorigenic osteosarcoma osteoblast-like cells, *in vitro*	+		25 µg/mL (d_{50} = 1.5 µm)	Miller *et al.* (2001)
Micronucleus formation, human non-tumorigenic osteosarcoma osteoblast-like cells, *in vitro*	+		5 mg/mL (d_{50} = 1–5 µm)	Miller *et al.* (2002)
Cell transformation (foci), human non-tumorigenic osteosarcoma osteoblast-like cells, *in vitro*	+		50 µg/mL (d_{50} = 1–5 µm)	Miller *et al.* (2001)
Cell transformation (foci), human non-tumorigenic osteosarcoma osteoblast-like cells, *in vitro*	+		10 mg/mL (d_{50} = 1–5 µm)	Miller *et al.* (2002)
Cobalt-containing metal carbides				
Cr_3C_2-Co				
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes *in vitro*	?		0.6 µg Co eq./mL	De Boeck *et al.* (2003b)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	e[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+		3 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	e[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mo_2C-Co				
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	?		0.6 µg Co eq./mL	De Boeck *et al.* (2003b)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	s[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	–		6 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	s[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)
NbC-Co				
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	?		0.6 µg Co eq./mL	De Boeck *et al.* (2003b)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	s[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+		3 µg Co eq./mL	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	e[d]		6 µg Co eq./mL	De Boeck *et al.* (2003b)
WC-Co				
Induction of FPG-sensitive sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	–[e]		6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (1998)
DNA breaks, alkaline elution, alkaline Comet assay, human lymphocytes, *in vitro*	+[e] s[e]		1.5 µg Co eq/mL (Co d_{50} = 4 µm) (WC d_{50} = 2 µm)	Anard *et al.* (1997)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human lymphocytes, *in vitro*	+[e]		3 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = 2 µm)	Anard *et al.* (1997)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in* vitro	+[e]		0.6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	Van Goethem *et al.* (1997)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	e[d]		6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	Van Goethem *et al.* (1997)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	+[c]		0.3 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	De Boeck *et al.* (1998)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	e[d]		6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	De Boeck *et al.* (1998)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	?		0.6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	De Boeck *et al.* (2003b)
DNA single-strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	e[d]		6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+[c]		0.6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	Van Goethem *et al.* (1997)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	e[d]		3 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} = < 1 µm)	Van Goethem *et al.* (1997)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	+		0.6 µg Co eq./mL (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003b)
Micronucleus formation, binucleates, cytochalasin-B assay, human lymphocytes, *in vitro*	e[d]		6 µg Co eq./mL(Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003b)
DNA single-strand breaks or alkali-labile sites, alkaline Comet assay, male Wistar rat type II pneumocytes, *in vivo*	+		16.6 mg/kg, i.t. (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003c)
DNA single-strand breaks or alkali-labile sites, alkaline Comet assay, male Wistar rat BALF cells, *in vivo*	–		16.6 mg/kg i.t. (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003c)
DNA single-strand breaks or alkali-labile sites, alkaline Comet assay, Wistar male rat mononuclear leukocytes, *in vivo*	–		16.6 mg/kg i.t. (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003c)
Micronucleus formation, male Wistar rat type II pneumocytes, *in vivo*	+		16.6 mg/kg i.t. (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003c)
Micronucleus formation, cytochalasin-B assay, male Wistar rat lymphocytes, *in vivo*	–		49.8 mg/kg i.t. (Co d_{50} = 4 µm) (WC d_{50} < 1 µm)	De Boeck *et al.* (2003c)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
rWC-Co particles				
DNA breaks, alkaline elution, purified DNA (3T3 mouse cells)	+[c] e[d]		1 μg Co eq./mL (Co d_{50} = 4 μm) (WC d_{50} = 2 μm)	Anard *et al.* (1997)
DNA breaks, alkaline elution, purified DNA (3T3 mouse cells)	r[e]		1 μg Co eq./mL + Na formate (Co d_{50} = 4 μm) (WC d_{50} = 2 μm)	Anard *et al.* (1997)
DNA breaks, alkaline elution, human mononuclear lymphocytes, *in vitro*	+[c]		1.5 μg Co eq/mL (Co d_{50} = 4 μm) (WC d_{50} = 2 μm)	Anard *et al.* (1997)
Cobalt compounds				
Co(II) salts				
Cobalt(II) acetate				
Inhibition of repair of UV-induced pyrimidine dimers, nucleoid sedimentation, human HeLa S-3 cells, *in vitro*	+		100 μM	Snyder *et al.* (1989)
Enhancement of cell transformation by simian adenovirus SA7, Syrian hamster embryo cells, *in vitro*	+		0.2 mM	Casto *et al.* (1979)
DNA base damage (products of hydroxyl radical attack), female and male Fischer 344/NCr rats, *in vivo*	+ (kidney > liver > lung)		ip, single, 50 μM/kg	Kasprzak *et al.* (1994)
Cobalt(II) chloride				
Reduction of fidelity of DNA replication by substitution of Mg^{2+} *Escherichia coli* DNA polymerase, sea-urchin nuclear DNA polymerase, avian myeloblastosis virus DNA polymerase	+		1 mM [130 μg/mL]	Sirover & Loeb (1976)
Prophage induction, *Escherichia coli*	–		~ 320 μM[f] [415 μg/mL]	Rossman *et al.* (1984)
Escherichia coli WP2$_s$, inhibition of protein synthesis	+		6.25 μg/mL	Leitão *et al.* (1993)
Escherichia coli ABI886, inhibition of protein synthesis	+		6.25 μg/mL	Leitão *et al.* (1993)
Salmonella typhimurium TA100, reverse mutation	–		NG	Ogawa *et al.* (1986)
Salmonella typhimurium TA102, reverse mutation	–	–	40 ppm [40 μg/mL]	Wong (1988)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535, reverse mutation	–		NG	Arlauskas *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	–	–	40 ppm [40 µg/mL]	Wong (1988)
Salmonella typhimurium TA1537, reverse mutation	–		NG	Arlauskas *et al.* (1985)
Salmonella typhimurium TA1537, reverse mutation	–		1000 µmol/plate [130 000 µg/plate]	Ogawa *et al.* (1986)
Salmonella typhimurium TA1537, reverse mutation	+	–	40 ppm [40 µg/mL]	Wong (1988)
Salmonella typhimurium TA1538, reverse mutation	–		NG	Arlauskas *et al.* (1985)
Salmonella typhimurium TA98, reverse mutation	–		NG	Arlauskas *et al.* (1985)
Salmonella typhimurium TA98, reverse mutation	–		NG	Ogawa *et al.* (1986)
Salmonella typhimurium TA98, reverse mutation	+	–	40 ppm [40 µg/mL]	Wong (1988)
Salmonella typhimurium TA2637, reverse mutation	–		1000 µmol/plate [130 000 µg/plate]	Ogawa *et al.* (1986)
Salmonella typhymurium, TA97 preincubation assay	+		100 µM [13 µg/mL]	Pagano & Zeiger (1992)
Salmonella typhimurium, TA97 preincubation assay	i[d]		100 µM [13 µg/mL] + DEDTC 420 µM	Pagano & Zeiger (1992)
Escherichia coli SY1032/pKY241 transfected with pUB_3, *supF* tRNA locus, mutation	+		20 µM [2.6 µg/mL]	Ogawa *et al.* (1999)
Bacillus subtilis rec strains H17/M45, growth inhibition	–		[325 µg/plate]	Nishioka (1975)
Bacillus subtilis rec strain H17, growth inhibition	+		[325 µg/plate]	Kanematsu *et al.* (1980)
Saccharomyces cerevisiae SBTD-2B, 'petite' mutation, respiratory deficiency	+		2 mM [260 µg/mL]	Prazmo *et al.* (1975)
Saccharomyces cerevisiae, strain 197/2d, 'petite' mutation	+		4 mM [520 µg/mL]	Putrament *et al.* (1977)
Saccharomyces cerevisiae, strain 197/2d, erythromycin-resistant mutation	–		4 mM [520 µg/mL]	Putrament *et al.* (1977)
Saccharomyces cerevisiae, 'petite' mutation, respiratory deficiency	(+)		640 µg/mL	Egilsson *et al.* (1979)
Saccharomyces cerevisiae D7, *ilv* gene mutation	–		10 mM [1300 µg/mL]	Fukunaga *et al.* (1982)
Saccharomyces cerevisiae D7, trp gene conversion	+		10 mM [1300 µg/mL]	Fukunaga *et al.* (1982)
Saccharomyces cerevisiae D7, *ilv* gene mutation	–		100 mM [13 000 µg/mL]	Singh (1983)
Saccharomyces cerevisiae D7, trp gene conversion	(+)		100 mM [13 000 µg/mL]	Singh (1983)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae D7, trp gene conversion	(+)		1500 µg/mL [11.5 mM]	Kharab & Singh (1985)
Saccharomyces cerevisiae D7, *ilv* gene mutation	(+)		3000 µg/mL [23 mM]	Kharab & Singh (1985)
Saccharomyces cerevisiae D7, 'petite' mutation, respiratory deficiency	+		750 µg/mL [5.76 mM]	Kharab & Singh (1987)
Drosophila melanogaster, gene mutation or mitotic recombination, wing spot test mwh/flr	+		2 mM [260 µg/mL]	Ogawa *et al.* (1994)
Drosophila melanogaster, gene mutation or reduced mitotic recombination, wing spot test mwh/TM3	–		8 mM [1040 µg/mL]	Ogawa *et al.* (1994)
DNA strand breaks, alkaline sucrose gradient, Chinese hamster ovary cells, *in vitro*	+		2 mM [260 µg/mL]	Hamilton-Koch *et al.* (1986)
DNA strand breaks, nucleoid sedimentation assay, Chinese hamster ovary cells, *in vitro*	–		10 mM [1300 µg/mL]	Hamilton-Koch *et al.* (1986)
DNA strand breaks, nucleoid sedimentation, human HeLa cells, *in vitro*	+		50 µM [65 µg/mL]	Hartwig *et al.* (1990)
DNA-protein cross links, rat Novikoff ascites hepatoma cells, *in vitro*	+		1 mM [130 µg/mL]	Wedrychowski *et al.* (1986)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus, *in vitro*	(+)		0.2 mM [26 µg/mL]	Miyaki *et al.* (1979)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus, *in vitro*	+		100 µM [13 µg/mL]	Hartwig *et al.* (1990)
Gene mutation, Chinese hamster V79 cell line, *Gpt* locus, *in vitro*	–		100 µM [13 µg/mL]	Kitahara *et al.* (1996)
Gene mutation, Chinese hamster transgenic cell line G12, *Gpt* locus, *in vitro*	+		50 µM [6.5 µg/mL]	Kitahara *et al.* (1996)
Sister chromatid exchanges, mouse macrophage-like cells P388D_1, *in vitro*	+		100 µM [13 µg/mL]	Andersen (1983)
Cell transformation, C3H10T1/2 mouse fibroblast cells, *in vitro*	+[c]		38 µM [5 µg/mL]	Doran *et al.* (1998)
Reduction in colony forming, V79 Chinese hamster cells, *in vitro*	+ (42%)		180 µM [24 µg/mL]	Kasten *et al.* (1992)
Reduction of cloning efficiency, Chinese hamster ovary cells, *in vitro*	+ (50%)		4 mM [520 µg/mL]	Hamilton-Koch *et al.* (1986)
Displacement of acridine orange from DNA, calf thymus DNA and *Micrococcus luteus* DNA	+		0.33 mM [43 µg/mL]	Richardson *et al.* (1981)
Formation of metal–DNA complex, calf thymus B-DNA	+		NG	Aich *et al.* (1999)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Induction of reporter gene expression under the control of the promoter region of the metallothionein gene, chick embryo liver cells transfected with luciferase or chloramphenicol acetyl transferase, *in vitro*	+		112 μM [15 μg/mL]	Lu *et al.* (1996)
Production of reactive oxygen species (fluorescence from oxidation of DCFH-DA), A549 cells, human lung cells, *in vitro*	+		200 μM [26 μg/ml]	Salnikow *et al.* (2000)
Production of reactive oxygen species (fluorescence from oxidation of DCFH-DA), A549 cells, human lung cells, *in vitro*	r[d]		300 μM [39 μg/mL] + 2-mercapto-ethanol	Salnikow *et al.* (2000)
Production of reactive oxygen species (fluorescence from oxidation of DCFH-DA), A549 cells, human lung cells, *in vitro*	r[d]		300 μM [39 μg/mL] + vitamin E	Salnikow *et al.* (2000)
DNA strand breaks, fluorescence analysis of DNA unwinding, human white blood cells, *in vitro*	+		50 μM [6.5 μg/mL]	McLean *et al.* (1982)
DNA strand breaks, alkaline sucrose gradient, human diploid fibroblasts, *in vitro*	+		5 mM	Hamilton-Koch *et al.* (1986)
DNA strand breaks, nick translation, human diploid fibroblasts, *in vitro*	+		10 mM [1300 μg/mL]	Hamilton-Koch *et al.* (1986)
DNA strand breaks, nucleoid sedimentation, human diploid fibroblasts, *in vitro*	–		10 mM [1300 μg/mL]	Hamilton-Koch *et al.* (1986)
DNA strand breaks and alkali-labile sites, alkaline Comet assay, human mononuclear leukocytes, *in vitro*	+[e]		0.3 μg/mL	De Boeck *et al.* (1998)
Induction of gene expression (*Cap43*), A549 cells, human lung cells, *in vitro*	+		100 μM [13 μg/mL]	Salnikow *et al.* (2000)
Induction of gene expression (*Cap43*), A549 cells, human lung cells, *in vitro*	s[d]		100 μM [13 μg/mL] + 2-mercapto-ethanol	Salnikow *et al.* (2000)
Induction of gene expression (*Cap43*), A549 cells, human lung cells, *in vitro*	s[d]		100 μM [13 μg/mL] + H_2O_2	Salnikow *et al.* (2000)
Sister chromatid exchange, human lymphocytes, *in vitro*	+		10 μM [1.3 μg/mL]	Andersen (1983)
Aneuploidy, human lymphocytes, *in vitro*	+		3.7 μg/mL	Resende de Souza-Nazareth (1976)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Aneuploidy, pseudodiploidy and hyperploidy, bone marrow of male hamsters, *in vivo*	+		400 mg/kg bw ip[g]	Farah (1983)
Aneuploidy, pseudodiploidy and hyperploidy, testes of hamsters, meiosis 1, *in vivo*	+		400 mg/kg bw ip[g]	Farah (1983)
Inhibition of binding of p53 protein to p53 consensus sequence on linear DNA fragment	+ (full)		> 100 µM (300 µM)	Palecek *et al.* (1999)
Inhibition of binding of p53 protein to supercoiled DNA	+		600 µM	Palecek *et al.* (1999)
Affinity of reconstituted apopolypeptide (Zn finger protein) with estrogen response element consensus oligonucleotide	r		NG (K_D 0.720 µM)[h]	Sarkar (1995)
Inactivation of bacterial Fpg protein (with Zn finger domain), conversion of supercoiled bacteriophage PM2 DNA into open circular form, electrophoresis	–		1000 µM	Asmuss *et al.* (2000)
Inhibition of XPA (with Zn finger domain) binding to UV-irradiated oligonucleotide, gel mobility shift analysis	+		50 µM [6.5 µg/mL]	Asmuss *et al.* (2000)
Cobalt(II) chloride hexahydrate				
Lysogenic induction, *Escherichia coli* $WP2_s$ (λ)	r[i]		(10 µg /mL)[f] + UV	Leitão *et al.* (1993)
Lysogenic induction, *Escherichia coli* K12 ABI886 (λ)	r[i]		(10 µg/mL)[f] + UV	Leitão *et al.* (1993)
Lysogenic induction, *Escherichia coli* ABI157 (λ)	+		100 µg/mL – Mg	Leitão *et al.* (1993)
Phage reactivation, *Escherichia coli* ABI157 (λ)	–		250 µg/mL – UV	Leitão *et al.* (1993)
Phage reactivation, *Escherichia coli* ABI157 (λ)	e[j]		62.5 µg/mL + UV	Leitão *et al.* (1993)
Escherichia coli WP2, reverse mutation	–[l]		20 µg/mL [84 µM]	Kada & Kamematsu (1978)
Escherichia coli WP2 *uvrA*, reverse mutation	–[l]		NG	Arlauskas *et al.* (1985)
Escherichia coli $WP2_s$ gene mutation	–[l]		50 µg/mL [210 µM]	Leitão *et al.* (1993)
Escherichia coli $WP2_s$ gene muation	r[l]		50 µg/mL + UV	Leitão *et al.* (1993)
Saccharomyces cerevisiae D7, 'petite' mutation, respiratory deficiency	+		[130 µg/mL]	Lindegren *et al.* (1958)
Salmonella typhimurium TA100, reverse mutation	–		100 mM [23 800 µg/mL]	Tso & Fung (1981)
Salmonella typhimurium TA100, reverse mutation	–		NG	Arlauskas *et al.* (1985)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1538, reverse mutation	–[f2]		20 µg/mL [84 µM]	Mochizuki & Kada (1982)
Salmonella typhimurium TA98, reverse mutation	–[f2]		20 µg/mL [84 µM]	Mochizuki & Kada (1982)
Bacillus subtilis strain NIG 1125, reverse mutation	–[f3]		30 µg/mL [126 µM]	Inoue *et al.* (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, *in vitro*	–		57.11 µg/mL	Amacher & Paillet (1980)
Gene mutation, Chinese hamster V79 cells, *8AG* locus, *in vitro*	–		9 µM [2 µg/mL]	Yokoiyama *et al.* (1990)
Gene mutation, Chinese hamster V79 cells, *8AG* locus, *in vitro*	r[j]		3 µM [0.7 µg/mL] + γ rays	Yokoiyama *et al.* (1990)
Micronucleus formation, BALB/c mouse bone marrow, *in vitro*	–	–	50 µg/mL [385 µM]	Suzuki *et al.* (1993)
DNA strand breaks, alkaline elution, human lymphocytes, *in vitro*	–		102 µM [25 µg/mL]	Anard *et al.* (1997)
Inhibition of nucleotide excision repair (incision and polymerization steps) of UV-induced DNA damage, alkaline unwinding, VH16 human fibroblasts	+		50 µM [12 µg/mL]	Kasten *et al.* (1997)
Inhibition of nucleotide excision repair (ligation step) of UV-induced DNA damage, alkaline unwinding, VH16 human fibroblasts	–		200 µM [48 µg/mL]	Kasten *et al.* (1997)
Inhibition of UV-induced cyclobutane pyrimidine dimers (incision step), alkaline unwinding + T4 endonuclease V, VH16 human fibroblasts	+		150 µM [86 µg/mL]	Kasten *et al.* (1997)
Micronucleus formation, male BALB/c AnNCrj mouse bone marrow, *in vivo*	+		50 mg/kg bw	Suzuki *et al.* (1993)
Micronucleus formation, male BALB/c AnNCrj mouse bone marrow, *in vivo*	e[i]		50 mg/kg + DMH 20 mg/kg	Suzuki *et al.* (1993)
Micronucleus formation, male BALB/c AnNCrj mouse bone marrow, *in vivo*	e[i]		50 mg/kg + benzo(a)pyrene 50 mg/kg	Suzuki *et al.* (1993)
Micronucleus formation, male BALB/c AnNCrj mouse bone marrow, *in vivo*	e[i]		50 mg/kg + 2-naphthylamine 200 mg/kg	Suzuki *et al.* (1993)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt(II) molybdenum(VI) oxide				
Enhancement of cell transformation by simian adenovirus SA7, Syrian hamster embryo cells, *in vitro*	+		250 µM [55 µg/mL]	Casto *et al.* (1979)
Cobalt(II) nitrate				
Chromosome aberrations (numerical), human diploid fibroblasts WL38 and MRC_5, *in vitro*	–		0.08 µM[g] [0.015 µg/mL]	Paton & Allison (1972)
Chromosome aberrations (numerical), human mononuclear leucocytes, *in vitro*	–		0.8 µM[g] [0.15 µg/mL]	Paton & Allison (1972)
Cobalt(II) nitrate hexahydrate				
Drosophila melanogaster (*flr³/In(3LR)TM3, ri p^p Sep bx^34e e^5 Ser*) × (*mwh*).*mwh* and *fbr³*, gene mutations, chromosomal deletion, non disjunction or mitotic recombination (small single spots and large single spots), SMART test	+		1 mM [291 µg/mL]	Yeºilada (2001)
Drosophila melanogaster (*flr³/In(ELR)TM3, ri p^p Sep bx^34e e^5 Ser*) × (*mwh*).*mwh and fbr³*, mitotic recombination (twin spots), SMART test	+		10 mM [2910 µg/mL]	Yeºilada (2001)
Cobalt(II) sulfate				
Allium cepa, chromosomal aberrations	+		20 µM [3 µg/mL]	Gori & Zucconi (1957)
Allium cepa, aneuploidy	+		100 µM [15 µg/mL] for 5 days + H_2O for 3 days	Gori & Zucconi (1957)
Production of reactive oxygen species (degradation of 2-deoxyribose), malondialdehyde assay	+		1 µM [0.155 µg/mL]	Ball *et al.* (2000)
Production of reactive oxygen species (degradation of 2-deoxyribose), malondialdehyde assay	r[d]		50 µM [7.8 µg/mL] + desferrioxamine 1 mM	Ball *et al.* (2000)
Chemical changes in DNA bases GC/MS-SIM, calf thymus DNA	–		25 µM [4 µg/mL]	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, calf thymus DNA	+[d]		25 µM [4 µg/mL] + H_2O_2 208 mM	Nackerdien *et al.* (1991)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtilis rec strain H17, growth inhibition	(+)		388 µg/plate	Kanematsu *et al.* (1980)
Cytoskeletal perturbation of microtubules and microfilaments, mouse cells SWISS 3T3, *in vitro*	+		100 µM [15.5 µg/mL]	Chou (1989)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	–		25 µM [4 µg/mL]	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	+[d]		25 µM + H_2O_2 208 mM	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	f[m]		25 µM [4 µg/mL] + H_2O_2 208 mM + EDTA 120 µM	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	f[m]		25 µM + H_2O_2 208 mM + mannitol 50 mM	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	f[m]		25 µM + H_2O_2 208 mM + DMSO 50 mM	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	f[m], e[m]		25 µM + H_2O_2 208 mM + glutathione 1 mM	Nackerdien *et al.* (1991)
Chemical changes in DNA bases GC/MS-SIM, isolated human chromatin, K562 cells	e[m]		25 µM + H_2O_2 208 mM + SOD 200 units/mL	Nackerdien *et al.* (1991)
Induction of human metal-inducible genes (*MT-IIA, hsp70, c-fos*), HeLa human cervical carcinoma cells, *in vitro*	+[n]		500 µM	Murata *et al.* (1999)
Metal responsive element (MRE)-DNA binding activity, HeLa human cervical carcinoma cells, *in vitro*	–		500 µM	Murata *et al.* (1999)
Heat shock element (HSE)-DNA binding activity, HeLa human cervical carcinoma cells, *in vitro*	?		500 µM	Murata *et al.* (1999)
Cobalt(II) sulfate monohydrate				
Cell transformation, Syrian hamster embryo cells, *in vitro*	+		0.125 µg/mL [0.75 µM]	Kerckaert *et al.* (1996)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cobalt(II) sulfate heptahydrate				
Salmonella typhimurium TA100, reverse mutation	+	–[o]	100 µg/plate	Zeiger *et al.* (1992)
Salmonella typhimurium TA98, TA1535, reverse mutation	–	–	10 000 µg/plate	Zeiger *et al.* (1992)
Induction of p53, ELISA assay, NCTC929 mouse fibroblasts, *in vitro*	+		50 µg/mL [178 µM]	Duerksen-Hughes *et al.* (1999)
CO(II)acetate tetrahydrate				
Chromosomal aberrations, human lymphocytes, *in vitro*	–		0.6 µg/mL [2.4 µM]	Voroshilin *et al.* (1978)
Co(III)hexaamine ions and Co(III) amine complexes				
Conformational changes of DNA oligonucleotides, circular dichroism and NMR spectroscopy	+		µM range (< 24 µM)	Bauer & Wang (1997)
Co(III) complexes				
Escherichia coli, strains AB1157 (wild type), AB1886 *uvr*A6, GW801 *rec*A56, GW802 *rec*56 *uvr*A6, GW803 *rec*A56 *lex*A⁻, PAM 5717 *lex*A and AB1899 *lon*, DNA repair assay	+ (8/15)[p]		NG	Schultz *et al.* (1982)
	(+) (7/15)[p]		NG	
Salmonella typhimurium, strain TA100, TA98, TA92, reverse mutation	+ (4/15)		0.1–0.5 µmol/plate	Schultz *et al.* (1982)
Salmonella typhimurium, strain TA1535, 1537, 1538. reverse mutation	–		2 µmol/plate	Schultz *et al.* (1982)
Co(III) salts				
$CoN0_3$				
Pisum abyssinicum chlorophyll mutation	+[q]		0.1–1 mM [18.3–183 µg/mL]	von Rosen (1964)
Co(III) complexes with desferal				
Plasmid PBR322, scission of double-stranded DNA	+		≤ 42.5 µM[r] + H_2O_2 4 mM	Joshi & Ganesh (1992)
$Co(OH)_3$				
Bacillus subtilis rec strain H17, growth inhibition	(+)		[2750 µg/plate]	Kanematsu *et al.* (1980)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Co(III) Schiff–base complex				
Inhibition of Zn-finger transcription factor, HNMR spectroscopy	+		0.5 mM	Louie & Meade (1998)
Inhibition of Zn-finger transcription factor, Sp1, gel shift, filter binding assay	+		10 μM	Louie & Meade (1998)
Cobalt sulfides (2^+) and (4^+)				
CoS particles				
DNA strand breaks, alkaline sucrose gradient, Chinese hamster CHO cells, *in vitro*	+		10 μg/mL	Robison *et al.* (1982)
Gene mutation, Chinese hamster transgenic cell line G10, *Gpt* locus, *in vitro*	–		1 μg/cm^2	Kitahara *et al.* (1996)
Gene mutation, Chinese hamster transgenic cell line G10, *Gpt* locus, *in vitro*	s[d]		1 μg/cm^2 + H_2O_2 10 μM	Kitahara *et al.* (1996)
Gene mutation, Chinese hamster transgenic cell line G12, *Gpt* locus, *in vitro*	+		0.5 μg/cm^2	Kitahara *et al.* (1996)
Gene mutation, Chinese hamster transgenic cell line G12, *Gpt* locus, *in vitro*	s[d]		0.5 μg/cm^2 + H_2O_2 10 μM	Kitahara *et al.* (1996)
CoS (amorphous)				
Cell transformation, Syrian hamster embryo cells, *in vitro*	(+)		10 μg/mL (d_{50} = 2.0 μm)	Abbracchio *et al.* (1982); Costa *et al.* (1982)
CoS_2 (crystalline)				
Cell transformation, Syrian hamster embryo cells, *in vitro*	+[g]		1 μg/mL (d_{50} = 1.25 μm)	Abbracchio *et al.* (1982); Costa *et al.* (1982)

Table 15 (contd)

rW-Ni-Co alloy, reconstituted mixture of W (92%), Ni (5%) and Co (3%) particles; rWC-Co, reconstituted mixture of WC (94%) and Co (6%) particles; HNMR, proton nuclear magnetic resonance

[a] ?, inconclusive; +, positive; (+), weak positive; –, negative; r = reduction; e = enhancement; s = stable

[b] LED, lowest effective dose; HID, highest ineffective dose; ip, intraperitoneally; po, orally; i.t., intratracheal instillation; MMS, methylmethane sulfonate; DMSO, dimethylsulfoxide; EDTA, ethyldiaminetetraacetate; DEDTC, diethyldithiocarbamate; NG, not given; DCFH-D, 2′,7′-dichlorofluorescine diacetate; DMH, 1,1-dimethylhydrazine; 8AG, 8-azaguanine; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; UV, ultraviolet irradiation

[c] Refer to the same experiment where Co and WC–Co were compared

[d] as compared to CO

[e] as compared to rWC-Co

[f] Estimated from a graph in the paper

[g] Total dose given to each animal over nine days

[h] This value corresponds to the dissociation constant (K_D) for cobalt-reconstituted polypeptide binding with estrogen response element consensus oligonucleotide

[i] as compared to the other mutagen used

[j] toxic dose; highest ineffective subtoxic dose was not given.

[k] Similar effect to strain *E. coli* WP2s(λ), but data not shown in the paper

[$I^{1, 2 \text{ or } 3}$] antimutagenic effect; [I^1], inhibition of mutagenesis induced by *N*-methyl-*N'*-nitrosoguanidine (MNNG); [I^2], inhibition of mutagenesis induced by 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) or [I^3], inhibition of spontaneous mutability

[m] as compared to Co + H_2O_2

[n] metallothionein (*MT-IIA*) and heat shock protein (*hsp70*) genes were induced but not *c-fos* gene.

[o] Tested at doses up to 10 000 μg/plate

[p] The ratio corresponds to the number of Co(III) complexes positive for DNA repair assay on the total number of Co(III) complexes tested

[q] Co as EDTA chelate (Co-EDTA) was also positive.

[r] Optimal concentration for 100% DNA cleavage; slight increase in concentration over this value lead to extensive degradation.

[s] more than corresponding amorphous salt

Hartwig & Schwerdtle, 2002) and with zinc finger proteins (Hartwig, 2001) and their effect on gene expression (Beyersman, 2002) have been reviewed. An evaluation of carcinogenic risks of cobalt and cobalt compounds was published in 1991 (IARC, 1991).

Metallic cobalt particles (median diameter (d_{50}) = 4 μm) have been shown with alkaline elution technology to induce DNA breakage and/or alkali-labile sites in DNA purified from 3T3 mouse cells. Similar changes have been demonstrated *in vitro* in human mononuclear leukocytes by both the alkaline elution and the Comet assay methods. Oxidative DNA damage was not detected at FPG-sensitive sites with the Comet methodology. In experiments run in parallel, a statistically-significant induction of micronuclei in binucleated human lymphocytes was obtained with the cytochalasin-B method. In-vitro cell transformation was not induced in mouse fibroblast cells by cobalt particles ($d_{50} \leq 5$ μm) nor in human osteoblast-like cells by approximately same size (d_{50} = 1–4 μm) cobalt particles.

Metallic cobalt (d_{50} = 1–5 μm) has been tested in combination with tungsten and nickel particles. *In vitro*, the mixture induced DNA single-strand breaks as shown by alkaline elution methodology, micronuclei, and cell transformation in human non-tumorigenic osteosarcoma osteoblast-like cell line (TE85, clone F-5).

(*b*) *Hard-metal particles*

When tested *in vitro* over a range of cobalt equivalent concentrations, a mixture of tungsten carbide and cobalt metal (WC–Co), caused significantly more (on average threefold more) DNA breaks than cobalt particles alone, both in isolated human DNA and in cultured human lymphocytes (alkaline elution and Comet assays); this DNA damage was inhibited by scavenging activated oxygen species. In the same assay run in parallel, cobalt chloride did not cause DNA breaks. Dose-dependency and time-dependency of DNA breakage and of induction of alkali-labile sites were shown for hard-metal particles in the Comet assay (De Boeck *et al.*, 2003b). A similarly greater genotoxic activity of hard metal compared with cobalt-metal particles alone has been found with the cytokinesis-blocked micronucleus test when applied *in vitro* to human lymphocytes. The data demonstrate clearly that interaction of cobalt with tungsten carbide particles leads to enhanced mutagenicity. Recently, this observation has been extended to other carbides. In the in-vitro cytokinesis-blocked micronucleus test, while the metal carbides alone did not increase the micronucleus frequency, cobalt alone and the four tested carbide–cobalt mixtures induced statistically-significant concentration-dependent increases in micronucleated binucleates. As with the tungsten carbide–cobalt metal mixture, nobium carbide and chromium carbide particles were able to interact with cobalt, producing greater mutagenic effects than those produced by the particles of the individual metals. Molybdenum carbide particles did not display interactive mutagenicity with cobalt in the micronucleus test, possibly because of their small specific surface area, compactness and/or spherical shape (De Boeck *et al.*, 2003b). However, with the Comet assay, when also performed directly at the end of the treatment, no firm conclusion could be made.

From a mechanistic point of view, the in-vitro studies comparing the effects of cobalt metal alone and the hard-metal mixture (WC–Co) provide convincing evidence that the

mutagenic activity of metallic cobalt is not exclusively mediated by the ionic form dissolved in biological media (Anard *et al.*, 1997). However, the dissolved cations do play an important role through direct or indirect mutagenic effects as reviewed separately for the soluble Co(II) and Co(III) compounds.

In-vivo experimental data on the mutagenicity of cobalt particles alone are lacking. Evidence of the in-vivo mutagenic potential of hard-metal dust was obtained recently in type II pneumocytes of rats (De Boeck, 2003c). DNA breaks/alkali-labile sites (alkaline Comet assay) and chromosome/genome mutations (micronucleus test) were assessed after a single intratracheal instillation of hard metal (WC–Co), and dose–effect and time trend relationships were examined. In addition, the alkaline Comet assay was performed on cells obtained from BALF and on peripheral blood mononucleated cells (PBMC). Protein content, LDH activity, total and differential cell counts of BALF were evaluated in parallel as parameters of pulmonary toxicity. In type II pneumocytes, WC–Co induced a statistically-significant increase in tail DNA (12-h time point) and in micronuclei (72 h) after a single instillation in rats at a dose which produced mild pulmonary toxicity. In PBMC, no increase in DNA damage nor in micronuclei was observed.

Cobalt compounds, like other metallic compounds, are known to be relatively inactive in prokaryotic systems (Rossman, 1981; Swierenga *et al.*, 1987).

(*c*) *Cobalt(II) chloride*

Cobalt(II) chloride was found to be inactive in the λ prophage induction assay, and gave conflicting results in the *Bacillus subtilis* $rec^{+/-}$ growth inhibition assay; when a cold preincubation procedure was used, positive results were observed (Kanematsu *et al.*, 1980). Lysogenic induction and phage reactivation was found in *Escherichia coli* in the absence of magnesium. Also in *E. coli*, reduction of fidelity of DNA replication by substitution of magnesium and inhibition of protein synthesis were observed. Cobalt(II) chloride was inactive in all but two bacterial mutagenicity tests. One study gave positive results in the absence, but not in the presence, of an exogenous metabolic system, and in the second study, a preincubation procedure was used.

In bacteria, cobalt(II) chloride has been reported to reduce the incidence of spontaneous mutations and to inhibit mutations induced by *N*-methyl-*N'*-nitrosoguanidine and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole. It was found to be comutagenic with several heteroaromatic compounds such as benzo(a)pyrene and naphthylamine.

In *Saccharomyces cerevisiae*, cobalt(II) chloride induced gene conversion and petite ρ^- mutation in mitochondrial DNA but not other types of mutation.

In *Drosophila melanogaster*, mitotic recombination was found.

In mammalian cells cultured *in vitro*, positive results were obtained for induction of DNA–protein cross-linkage, DNA strand breakage and sister chromatid exchange in most studies. Cobalt(II) chloride induced mutations at the *Hprt* locus in Chinese hamster V79 cells, but not at the *8AG* and the *Gpt* loci. At the same *Gpt* locus in a transgenic Chinese hamster V79 G12 cell line, lower concentrations of cobalt(II) chloride did induce gene

mutations. In a single study, at the *Tk* locus in mouse lymphoma L5178Y cells, the results were negative.

In most studies, in cultured human cells *in vitro*, positive results were obtained for inhibition of protein-DNA binding activities, inhibition of p53 binding to DNA and for induction of gene expression, induction of DNA strand breakage and sister chromatid exchange. Chromosomal aberrations were not observed in cultured human cells (IARC, 1991). [The Working Group noted the low concentrations employed.] Cobalt(II) chloride induced aneuploidy in cultured human lymphocytes.

In vivo, cobalt(II) chloride administered by intraperitoneal injection induced aneuploidy (pseudodiploidy and hyperploidy) in bone marrow and testes of Syrian hamsters, micronuclei in bone marrow in male BALB/c mice, and enhanced the micronuclei frequencies induced by the three other mutagens tested.

A gene expression mechanism is involved in several tissue and cellular responses induced by soluble cobalt (generally cobalt chloride) mimicking the pathophysiological response to hypoxia, a response which involves various genes including those coding for erythropoiesis and for growth factors for angiogenesis (Gleadle *et al.*, 1995; Steinbrech *et al.*, 2000; Beyersmann, 2002). Up-regulation of erythropoietin gene expression was observed *in vivo* after a single intraperitoneal injection of cobalt chloride (60 mg/kg bw) into rats (Göpfert *et al.*, 1995) and might be of relevance in explaining the polyglobulia noted in humans treated with high doses of cobalt (Curtis *et al.*, 1976). In Chinese hamster ovary cells, cobalt also up-regulated the expression of haeme oxygenase-1, a potent antioxidant and anti-inflammatory mediator which helps to maintain cellular homeostasis in response to stress and injury (Gong *et al.*, 2001).

In studies designed to explore the molecular mechanisms of gene response to hypoxia, cobalt (12 and 60 mg/kg bw as cobalt chloride) was found to up-regulate the expression of the *PDGF-B* gene in lungs and kidneys of male Sprague-Dawley rats (Bucher *et al.*, 1996). Since PDGF is an important growth factor which modulates cell proliferation and the expression of several proto-oncogenes mainly in mesenchymal cells, this effect of cobalt might explain how it may exert fibrogenic and/or carcinogenic properties, but this remains to be documented.

(*d*) *Other cobalt compounds*

Few results are available with other cobalt(II) salts.

Molecular analysis of lung neoplasms of B6C3F$_1$ mice exposed to cobalt sulfate heptahydrate showed the presence of K-*ras* mutations with a much higher frequency (55%) of G > T transversion at codon 12 than in controls (0%). This provides suggestive evidence that cobalt sulfate heptahydrate may indirectly damage DNA by oxidative stress (National Toxicology Program, 1998).

Cobalt sulfate has been shown to induce chromosomal aberrations and aneuploidy in plant cells, chemical changes in bases in purified calf thymus DNA and in isolated human chromatin in the presence of hydrogen peroxide, and cytoskeletal perturbation of micro-

tubules and microfilaments and p53 protein in mouse fibroblasts treated *in vitro*. Cell transformation of Syrian hamster embryo cells has been induced by cobalt sulfate *in vitro*.

A number of mammalian genes (metallothionein MT-IIA, heat-shock proteins hsp70, c-fos) are transcriptionally regulated by a *cis*-acting DNA element located in their upstream regions. This DNA element responds to various heavy metals, including cobalt, to stimulate the expression of these genes (Murata *et al.*, 1999). *MT-IIA* and *hps70* but not *c-fos* RNA transcripts were increased in HeLa S_3 cells exposed to high concentrations of cobalt sulfate (> 10 μM). Metal response element (MRE)-DNA binding activity was not inhibited by cobalt sulfate in Hela cells *in vitro* while the results for heat shock element (HSE)-DNA binding activity were inconclusive. It is unknown whether MT-IIA and hps70 induction plays a role in the pathophysiological processes involved in cobalt carcinogenesis.

Cobalt(II) acetate was found to induce cell transformation *in vitro*. Cobalt(II) acetate and cobalt(II) molybdenum(VI) oxide ($CoMoO_4$) enhanced viral transformation in Syrian hamster embryo cells. Cobalt(II) acetate was shown to induce DNA base damage in female and male Fischer 344/NCr rats. Cobalt sulfide particles were found to induce DNA strand breaks and alkali-labile sites in Chinese hamster ovary cells. Data on the induction of gene mutations in Chinese hamster cells by cobalt sulfide particles are conflicting. Cobalt sulfide was shown to induce morphological transformation in Syrian hamster embryo cells; the crystalline form of cobalt sulfide being more active than the amorphous form.

Cobalt(III) nitrate induced gene mutations in *Pisum abyssinicum* chlorophyll. Eight of 15 cobalt(III) complexes with aromatic ligants were found to be positive in a DNA repair assay and four among the eight were also mutagenic to *Salmonella typhimurium*. Cobalt(III) complexes with desferal-induced scission of double-stranded DNA, and a cobalt(III) Schiff-base complex induced inhibition of zinc-finger transcription factors.

4.5 Mechanistic considerations

It had been assumed that, as for other metals, the biological activity of cobalt-metal particles, including their genotoxic effects, were mediated by the ionic form of cobalt and could be revealed by testing soluble compounds. However, Lison *et al.* (1995) demonstrated *in vitro* that cobalt metal, and not its ionic (II) species, was thermodynamically able to reduce oxygen in ROS independently of the Fenton reaction. During this process, soluble cobalt ions are produced which have several major cellular targets for induction of genotoxic effects and may, in turn, take part in a Fenton reaction in the presence of hydrogen peroxide. Moreover, since metallic cobalt forms particles which can be inhaled, assessment of genetic effects should also take into consideration: (i) that the primary production of ROS is related to the specific surface properties of the particles or the presence of transition metals, together with other parameters such as particle size, shape and uptake; and (ii) that excessive and persistent formation of ROS by inflammatory cells can lead to secondary toxicity. Since the mechanisms leading to the genotoxic effects of metallic cobalt are complex, assessment of its mutagenic effects should not be restricted

to the genetic effects of metallic cobalt alone but should be complemented by those of cobalt in association with carbides, and of cobalt salts.

The results of genotoxicity assays with cobalt salts demonstrate clearly their mutagenic potential. Recent experimental studies have contributed to better delineate the molecular mechanisms involved in the genotoxic (and carcinogenic potential) of cobalt ions. These mechanisms may conceivably apply both to soluble cobalt compounds — for example, cobalt chloride or sulfate — and also to cobalt-metal or hard-metal particles which are readily solubilized in biological media. *In vivo*, however, the bioavailability of cobalt(II) is relatively limited because these cations precipitate in the presence of physiological concentrations of phosphates ($Co_3(PO_4)_2$); K_s: 2.5×10^{-35} at 25 °C) and bind to proteins such as albumin.

In vitro in mammalian cells, two mechanisms seem to apply :

(1) a direct effect of cobalt(II) ions causing damage to DNA through a Fenton-like mechanism;

(2) an indirect effect of cobalt(II) ions through inhibition of repair of DNA damage caused by endogenous events or induced by other agents.

In vitro, cobalt(II) has been shown to inhibit the excision of UV-induced pyrimidine dimers from DNA in a dose-dependent fashion. Inhibition of repair by cobalt(II) resulted in the accumulation of long-lived DNA strand breaks suggesting a block in the gap-filling stage (DNA polymerization) of repair. Ability to inhibit repair was not correlated with cytotoxicity. It has been shown that repair of X-ray-induced DNA damage is not sensitive to cobalt. All inhibitory metals inhibited closure of single-strand DNA breaks (Snyder *et al.*, 1989).

In vitro, ionic cobalt(II) was shown to inhibit nucleotide excision repair processes after ultraviolet (UV) irradiation as measured by the alkaline unwinding method. A concentration as low as 50 μM cobalt chloride inhibited the incision as well as the polymerization step of the DNA repair process in human fibroblasts treated with UV light. As the repair of DNA damage is an essential homeostatic mechanism, its inhibition may account for a mutagenic or carcinogenic effect of cobalt(II) ions. Concentrations less than 1 mM cobalt chloride did not affect the activity of bacterial fpg but significantly reduced the DNA binding activity of the mammalian damage recognition protein XPA. Competition with essential magnesium ions and binding to zinc finger domains in repair proteins have been identified as potential modes of indirect genotoxic activity of cobalt(II) ions. It has also been reported that the DNA binding activity of the p53 protein, which is a zinc-dependent mechanism, can be modulated by cobalt(II) ions (Kasten *et al.*, 1997; Palecek *et al.*, 1999; Asmuss *et al.*, 2000).

This indirect mutagenic effect of cobalt on repair enzymes is not restricted to cobalt salts but has been shown to apply also to in-vitro exposure to metallic cobalt. De Boeck *et al.* (1998) examined the effects of cobalt-metal particles using the alkaline Comet assay on methyl methanesulfonate (MMS)-treated isolated human lymphocytes. MMS induced DNA strand breaks and alkali-labile sites in the lymphocytes in a dose-dependent manner. Post-incubation of MMS-treated cells for 2 h, in the absence of cobalt, resulted in signi-

ficantly less DNA damage, implying that repair took place. Post-treatment with cobalt particles at a non-genotoxic dose for 2 h, after treatment with 5.5 µg/mL MMS, resulted in higher damage values compared with post-incubation values. These results may reflect inhibition by the cobalt particles of the ongoing repair of MMS-induced DNA lesions, which had presumably reached the polymerization step. Simultaneous exposure of lymphocytes to 5.5 µg/mL MMS and 1.2 µg/mL cobalt for 2 h resulted in higher damage values, conceivably representing an interference of cobalt particles at the incision of methylated bases, allowing more alkali-labile apurinic sites to be expressed, which, in the absence of cobalt, would be repaired. The authors concluded that metallic cobalt could cause persistence of MMS-induced DNA lesions by interference during their repair.

Since the previous IARC evaluation of cobalt in 1991, additional information has been obtained on the genotoxicity of the various cobalt species.

Cobalt(II) ions have been shown to substitute for zinc in the zinc-finger domain of some important proteins, such as those controlling cell cycling and/or DNA repair processes in animal and human cells.

Cobalt-metal particles produce mutagenic effects *in vitro* by two different mechanisms:

- directly through the production of ROS resulting in DNA damage, and
- indirectly by releasing Co(II) ions which inhibit DNA repair processes.

Moreover, when cobalt-metal particles are mixed with metallic carbide particles (mainly tungsten carbide), they form a unique chemical entity which:

- produces higher amounts of ROS than cobalt alone *in vitro*,
- has a stronger mutagenic activity than cobalt alone *in vitro* in human cells, and
- is mutagenic in rat lung cells in *vivo*.

A physicochemical mechanism to explain this increased toxicity has been proposed.

In humans, a specific fibrosing alveolitis (so-called hard-metal disease) occurs in workers exposed to dusts containing metallic cobalt such as hard metal or cemented microdiamonds. Fibrosing alveolitis may be a risk factor for lung cancer in humans.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Cobalt is widely distributed in the environment, occurring in the earth's crust mainly in the form of sulfides, oxides and arsenides. Cobalt metal is used to make corrosion- and wear-resistant alloys used in aircraft engines (superalloys), in magnets (magnetic alloys) and in high-strength steels and other alloys for many applications. Cobalt metal is added to metallic carbides, especially tungsten carbide, to prepare hard metals (two-phase composites; also known as cemented carbides) for metal-working tools. Cobalt is also used to manufacture cobalt-diamond grinding tools, cobalt discs and other cutting and grinding tools made from cobalt metal. Other uses of cobalt compounds include catalysts, batteries, dyes and pigments and related applications. Occupational exposure to cobalt occurs pre-

dominantly during refining of cobalt, in the production of alloys, and in the hard-metal industry where workers may be exposed during the manufacture and maintenance of hard-metal tools and during the use of diamond-cobalt tools.

5.2 Human carcinogenicity data

Several reports addressing cancer risks among workers in hard-metal production facilities in France provide evidence of an increased lung cancer risk related to exposure to hard-metal dust containing cobalt and tungsten carbide. The risk appears to be highest among those exposed to unsintered rather than sintered hard-metal dust. There is evidence for an increasing lung cancer risk with increasing duration of exposure in analyses which took into account potential confounding by smoking and other occupational carcinogens.

An earlier and smaller study of workers exposed to cobalt and tungsten carbide in the hard-metal industry in Sweden found increased mortality from lung cancer in the full cohort, with a higher risk among those with longer duration of exposure and latency. The study provides limited confirmation due to the small number of exposed lung cancer cases, the lack of adjustment for other carcinogenic exposures and the absence of a positive relationship between intensity of exposure and lung cancer risk.

The study of workers in hard-metal factories in France also allowed estimation of lung cancer risk in relation to exposures to cobalt in the absence of tungsten carbide. A twofold increased lung cancer risk was observed. However, no exposure–response relationships were reported and the results were not adjusted for other occupational carcinogens or smoking. Another study in the cobalt production industry in France reported no increase in risk of lung cancer mortality among cobalt production workers, but the study was limited by very small numbers.

5.3 Animal carcinogenicity data

Cobalt sulfate heptahydrate as an aqueous aerosol was tested in a single study by inhalation exposure in male and female mice and rats. Increased incidences of alveolar/bronchiolar neoplasms were seen in both sexes of both species. There was also an increase in adrenal pheochromocytomas in female rats. It was uncertain whether a marginal increase in pheochromocytomas in male rats was caused by cobalt sulfate.

Cobalt metal powder was tested in two experiments in rats by intramuscular injection and in one experiment by intrathoracic injection, and in rabbits in one experiment by intraosseous injection. All the studies revealed sarcomas at the injection site.

A finely powdered cobalt–chromium–molybdenum alloy was tested in rats by intramuscular injection and produced sarcomas at the injection site. In two other experiments in rats, coarsely- or finely-ground cobalt–chromium–molybdenum alloy implanted in muscle, or pellets of cobalt–chromium–molybdenum alloy implanted subcutaneously, did not induce sarcomas. Implantation in the rat femur of three different cobalt-containing alloys, in the form of powder, rod or compacted wire, resulted in a few local sarcomas. In another

experiment, intramuscular implantation of polished rods consisting of three different cobalt-containing alloys did not produce local sarcomas. In an experiment in guinea-pigs, intramuscular implantation of a cobalt–chromium–molybdenum alloy powder did not produce local tumours.

Intraperitoneal injection of a cobalt–chromium–aluminium spinel in rats produced a few local malignant tumours, and intratracheal instillation of this spinel in rats was associated with the occurrence of a few pulmonary squamous-cell carcinomas.

Interpretation of the evidence available for the carcinogenicity of cobalt in experimental animals was difficult because many of the reports failed to include sufficient details on results of statistical analyses, on survival and on control groups. Furthermore, such statistical analyses could not be performed by the Working Group in the absence of specific information on survival including fatality due to the neoplasms. Nevertheless, in the evaluation, weight was given to the consistent occurrence of tumours at the site of administration and to the histological types of tumours observed. However, intramuscular or subcutaneous injection of relatively inert foreign materials into rats is known to result in malignant tumours at the injection site, therefore limiting the interpretation of the results.

5.4 Other relevant data

The absorption rate of inhaled cobalt-containing particles is dependent on their solubility in biological fluids and in macrophages. In humans, gastrointestinal absorption of cobalt has been reported to vary between 5 and 45% and it has been suggested that absorption is higher in women than in men. Cobalt can be absorbed through intact human skin. It does not accumulate in any specific organ, except in the lung when inhaled in the form of insoluble particles. High concentrations of cobalt in blood are found in workers exposed to cobalt, in uraemic patients and in persons taking multivitamin preparations. Most of the absorbed cobalt is excreted in the urine within days, but a certain proportion is eliminated slowly, with half-life values between 2 and 15 years. Cobalt ions bind strongly to circulating proteins, mainly albumin. Cobalt concentrations in blood and/or in urine can be used in biological monitoring to assess individual exposure. After inhalation of metallic cobalt particles with tungsten carbide, toxic effects (alveolitis, fibrosis) occur at the site of contact and deposition. These effects are caused by the particles themselves and by solubilized cobalt ions. Systemic effects outside the respiratory tract are unlikely to be due to the particles. The main non-malignant respiratory disorders caused by inhalation of metallic cobalt-containing particles are bronchial asthma (any cobalt compounds) and fibrosing alveolitis (cobalt metal mixed with tungsten carbide or with microdiamonds). Fibrosis alveolitis, also known as hard-metal lung disease, is characterized pathologically as a giant-cell interstitial pneumonia; there is no evidence that it is caused by cobalt metal alone or cobalt salts. Non-respiratory toxic effects of cobalt include stimulation of erythropoiesis, and toxicity in the thyroid and the heart. Cobalt has skin-sensitizing properties, which may lead to contact dermatitis or airborne dermatitis.

In animals, it has been demonstrated that the health status of the lung affects the rate of clearance and retention of cobalt-containing particles. Smaller particles show a higher dissolution rate than larger ones. When mixed with tungsten carbide, the absorption and subsequent excretion of intratracheally-instilled cobalt-metal particles is greatly enhanced.

In experimental animals, various cobalt compounds cause a variety of toxic effects in the respiratory tract (pulmonary oedema, acute pneumonia), thyroid, erythropoietic tissue, myocardium and reproductive organs. A mixture of cobalt-metal particles and tungsten carbide caused effects that were much more severe than those observed with cobalt metal alone. Specific surface chemistry and increased production of reactive oxygen species at the site of mutual contact between cobalt and tungsten carbide are likely to play a role in this phenomenon. Cobalt-metal particles are weak inducers of reactive oxygen species *in vitro*, but this effect is greatly enhanced by the presence of tungsten carbide particles.

Exposure by inhalation to cobalt oxide, cobalt chloride or cobalt sulfate gives rise to a spectrum of inflammatory and proliferative changes in the respiratory tract in animals. Biochemical effects include increased levels of oxidized glutathione and stimulation of the pentose phosphate pathway, both of which are indicative of oxidative stress.

Reproductive effects of cobalt chloride include teratogenic effects in mice, and growth retardation and reduced postnatal survival in rats. Decreased fertility, testicular weights and sperm concentration have also been observed in mice. Inhalation of cobalt sulfate also gave rise to decreased sperm motility and increased sperm abnormality in mice, but not in rats.

In vitro, cobalt has been shown to induce various enzymes involved in the cellular response to stress and to interfere with cell-cycle control.

The results of genotoxicity assays with a variety of cobalt salts demonstrate the mutagenic potential of these salts both *in vitro* and *in vivo*. Moreover, from experiments performed with a mixture of cobalt and tungsten carbide particles, there is strong evidence that the mixture is mutagenic *in vitro*. It was also demonstrated to be mutagenic *in vivo* in rat lung cells.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of cobalt metal with tungsten carbide.

There is *inadequate evidence* in humans for the carcinogenicity of cobalt metal without tungsten carbide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulfate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt-metal powder.

There is *limited evidence* in experimental animals for the carcinogenicity of metal alloys containing cobalt.

There is *inadequate evidence* in experimental animals for the carcinogenicity of cobalt–aluminum–chromium spinel.

Overall evaluation

Cobalt metal with tungsten carbide is *probably carcinogenic to humans (Group 2A)*.

A number of working group members supported an evaluation in Group 1 because: (1) they judged the epidemiological evidence to be sufficient, leading to an overall evaluation in Group 1; and/or (2) they judged the mechanistic evidence to be strong enough to justify upgrading the default evaluation from 2A to 1. The majority of working group members, who supported the group 2A evaluation, cited the need for either sufficient evidence in humans or strong mechanistic evidence in exposed humans.

Cobalt metal without tungsten carbide is *possibly carcinogenic to humans (Group 2B)*.

Cobalt sulfate and other soluble cobalt(II) salts are *possibly carcinogenic to humans (Group 2B)*.

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